

**Behavioural abnormalities and novel pharmaceutical intervention in acute
and developmental rat models of psychiatric illness**

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In the Department of Physiology
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By
Brittney R. Lins

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Dean
College of Graduate and Postdoctoral Studies
University of Saskatchewan
116 Thorvaldson Building, 110 Science Place
Saskatoon, Saskatchewan S7N 5C9
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ABSTRACT

This thesis explores various methods of modelling psychiatric illness in rats. I used two distinct pharmacological manipulations to induce acute states that resemble psychosis, as well as a prenatal manipulation via maternal inflammation to produce a neurodevelopmental model. I assessed the effects of these manipulations in a variety of behaviour tasks relevant to psychiatric illness.

The first experiment (Chapter 2) uses a prepulse inhibition (PPI) behaviour test to demonstrate the effects of two enantiomers of putative antipsychotic GOV on APO or MK-801 disrupted sensorimotor gating. The use of APO and MK-801 allow acute manipulation of DAergic and glutamatergic systems, respectively; the two neurotransmitter systems implicated in the main theories explaining schizophrenia pathology. The l-, but not d- enantiomer of GOV was effective in restoring PPI, an effect which may be due to l-GOV's dual effects of D2 receptor antagonism and increased prefrontal cortex (PFC) DA efflux while the d- enantiomer lacks a strong D2 antagonistic effect. These results support previous findings that l-GOV may have potential for use as an antipsychotic.

The second set of experiments are presented in Chapters 3 and 4 and use a neurodevelopmental model of maternal immune activation (MIA) during pregnancy. Pregnant dams are treated with a single intravenous injection of immune stimulant polyinosinic:polycytidylic acid (polyI:C) or saline on gestational day (GD) 15. In the acute phase following treatment (48 h), dams that received polyI:C displayed significantly lower body weight compared to controls and maternal serum collected 3 h post treatment contained elevated levels of immune cytokines IL-6 and CXCL1 as determined by ELISA. When the offspring were delivered, pups born to polyI:C-exposed dams were significantly smaller than control pups. Long-term follow-up of the adult male offspring in Chapter 3 show the polyI:C offspring display a psychiatric-like phenotype characterized by behaviour abnormalities related to the positive, negative and cognitive symptoms of schizophrenia. In particular, male polyI:C offspring were more sensitive to the locomotor-inducing effects of systemic MK-801 and had deficient social interaction behaviour compared to controls. Male polyI:C offspring were selectively impaired at visual and crossmodal memory, as well as oddity preference. Two operant tasks were also used

to assess cognition in the male offspring. Operant set-shifting and reversal learning used lever-equipped operant chambers to assess cognitive flexibility and found a selective facilitation in set shifting performance with no effect on initial visual cue learning or reversal learning. Testing with a touchscreen-based reversal learning task also showed no effect on initial cue learning but polyI:C offspring were impaired at reversal learning, specifically in the late phase.

The effects of MIA on the behaviour profile of the adult female offspring is highlighted in Chapter 4. The females were tested in the same behaviour battery as their male siblings, with the exception of the operant tasks. Female offspring displayed a similar schizophrenia-like behavior profile, including reduced social interaction, and impaired visual recognition memory. Females failed to show a group difference in locomotor response to MK-801 at the dose examined, and neither group could perform the crossmodal memory task. When the male and female results were analysed together in a sex by treatment design, no significant evidence for sex differences were found.

In addition to the behaviour effects seen in MIA offspring, we were interested in the possibility of a relationship between maternal serum cytokine levels and the severity of the behavioural abnormalities in the offspring. Bivariate correlations were conducted on these data, but no robust relationships were observed, suggesting that IL-6, TNF- α , and CXCL1 concentrations 3 h post polyI:C injection are not good indicators of later behaviour effects.

This thesis uses these 3 data chapters to highlight concepts of validity in preclinical research, and the challenges of modelling complex human psychiatric conditions in rats while comparing and contrasting the models used. Overall, a researcher's choice of model depends on many factors, yet both pharmacological and neurodevelopmental approaches achieve sufficient levels of validity to advance the understanding of psychiatric illness for the eventual goal of improved disease management.

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DEDICATION

This work is first dedicated to my mother Glenna, my father Brian, and my brother Blake. Thank you for your endless support, it is the reason I was able to pursue higher education.

To my family and friends who never knew what I was talking about, but supported me anyway, this is dedicated to you as well.

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LIST OF ABBREVIATIONS

AC	adenylate cyclase
AMPH	amphetamine
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
APO	apomorphine
Ca ²⁺	calcium ion
CMOR	crossmodal object recognition
CSPP	cortico-striato-pallido-pontine circuitry
CSF	cerebral spinal fluid
CXCL	ligand for CXC family of cytokine receptors
CXC	amino acid residue sequence; cystine, variable amino acid, cystine
	CXCL1 ligand 1 for CXC receptor
	CXCL2 ligand 2 for CXC receptor
CXCR	receptor for CXC ligands
D1	dopamine receptor 1
D2	dopamine receptor 2
D3	dopamine receptor 3
D4	dopamine receptor 4
D5	dopamine receptor 5
DA	dopamine
DSM-5	Diagnostic and Statistical Manual of Mental Disorders 5
EPSE	extrapyramidal side effects
EPSC	excitatory postsynaptic current
FGA	first-generation antipsychotics
GABA	γ -amino-butyric acid
GOV	govadine, putative antipsychotic with D2 antagonist and DA efflux effects
	d-GOV d enantiomer of govadine
	l-GOV l enantiomer of govadine
GPCR	G protein coupled receptor

IL	interleukin
	IL-1 β interleukin 1 beta
	IL-6 interleukin 6
	IL-8 interleukin 8
IPSC	inhibitory postsynaptic current
K ⁺	potassium ion
LPS	lipopolysaccharide
Mg ²⁺	magnesium ion
MIA	maternal immune activation
Na ⁺	sodium ion
NAc	nucleus accumbens
MK-801	non-competitive NMDA receptor antagonist, also known as dizocilpine
NIH	National Institute of Health
NIMH	National Institute of Mental Health
NMDA	N-methyl-D-aspartate
OFC	orbitofrontal cortex
PCP	phencyclidine; non-competitive NMDA receptor antagonist
PFC	prefrontal cortex
	dlPFC dorsolateral prefrontal cortex
	dmPFC dorsomedial prefrontal cortex
	mPFC medial prefrontal cortex
PKA	protein kinase A
PNN	perineuronal net
polyI:C	polyinosinic:polycytidylic acid
PPI	prepulse inhibition
RDoC	Research Domain Criteria
SGA	second-generation antipsychotics
SSD	Schizophrenia Spectrum Disorders
TGF β	transforming growth factor beta
TH	tyrosine hydroxylase

THPB	tetrahydroprotoberberine
TLR3	Toll-like receptor 3
TNF- α	tumor necrosis factor alpha
TRS	treatment resistant schizophrenia

CHAPTER 1

INTRODUCTION

1.1 Outline of the thesis

This thesis takes several approaches to explore pathology, putative treatment, and environmental risk factors of psychiatric illness. I use two pharmacological models and a neurodevelopmental model to demonstrate various aspects of psychopathology and disease risk factors in order to better understand the biological disruptions that result in the characteristic behavioural disturbances observed in patients with psychiatric conditions.

Psychiatric illnesses are often chronic, debilitating, and poorly understood (Winship et al., 2018). Treatment options are largely insufficient treatment and success is limited (Lieberman et al., 2005; Jones et al., 2006; Lally and MacCabe, 2015). Only 14% of patients show recovery within 5 years of the onset of psychotic episodes, and long-term studies show up to an additional 16% recover within 25 years (Insel, 2010). Schizophrenia is a developmental psychiatric illness with multiple symptom domains (positive, negative, and cognitive) and a classic example where increased risk has been linked to gestational adversity (Brown and Meyer, 2018). The symptoms of schizophrenia are diverse. The distinctive hallucinations and delusions associated with the disease are referred to as the positive symptoms, while the negative symptoms include social withdrawal, avolition, and anhedonia. Schizophrenia is also characterised by cognitive symptoms, exemplified by impairments in working memory, information processing, visual learning and memory, and reasoning and problem solving (Young et al., 2009; Winship et al., 2018). Subsets of the symptoms associated with schizophrenia also tend to be observed in other diagnoses. Notably, impaired social functioning is a hallmark of autism, and major depression is associated with avolition and anhedonia (Nestler et al., 2002; Silverman et al., 2010). The work outlined in this dissertation is largely framed in the context of schizophrenia, but the results may apply to the understanding and management of other psychiatric conditions and mood disorders with overlapping clinical presentations (Nestler and Hyman, 2010; Sanislow et al., 2010). The general goals of my doctoral research were to produce translationally relevant results using preclinical rat models to further the understanding mental illness, particularly the characteristic

behaviour abnormalities seen in schizophrenia. My first goal was to determine the effects of 2 enantiomers of govadine (GOV), a compound with potential antipsychotic effects, in 2 pharmacological models of acute psychosis using a sensorimotor gating behaviour task. My second goal was to determine whether acute elevations of relevant cytokines in maternal serum following an acute inflammatory event during pregnancy were correlated with the long-term behaviour abnormalities seen in their adult offspring. Additional attention was given to the presence of sex effects with the goal of determining whether offspring sex was a factor in the behaviour outcomes.

1.2 Relevant pharmacology

1.2.1 The dopamine hypothesis

The dopamine hypothesis proposes dysfunction of the DAergic system is the underlying cause of schizophrenia, and this remains the most persistent theory of the neurobiology of the disease. Dopamine (DA) is a neurotransmitter that binds metabotropic g-protein coupled receptors (GPCR). There are 5 subgroups of DA receptors. D1 and D5 are grouped together as D1-class and they activate $G\alpha_{s/olf}$ G proteins to increase cAMP production by adenylate cyclase (AC) and increase PKA activity, and D2, D3 and D4 are D2-class which activate $G\alpha_{i/o}$ for the opposite effect of inactive AC, reduced cAMP, and inactive PKA (Beaulieu and Gainetdinov, 2011). D1-class increase inhibitory postsynaptic current (IPSC) and D2-class increase excitatory postsynaptic current (EPSC). DA has important roles in many neurological functions, with movement and reward mechanisms among the most well-known. DA is implicated in numerous disorders and its role in schizophrenia is particularly relevant to this thesis.

The initial theories of heightened DA activity in schizophrenia were proposed due to several early lines of evidence. Typical antipsychotics such as haloperidol are effective at treating the positive symptoms of schizophrenia and these act as D2 receptor antagonists, suggesting some form of increased D2 receptor activity in disease pathology (Beaulieu and Gainetdinov, 2011). Patients with schizophrenia show enhanced sensitivity to treatment with DAergic agonists such as apomorphine (APO), amphetamine (AMPH) and methylphenidate (Seeman, 2013). AMPH stimulates the release of DA, while APO is a classic, nonspecific DA

receptor agonist with high affinity for D1-D5 receptors (Seeman, 2013; Arroyo-García et al., 2018; Horowski and Löschmann, 2019). Some studies show increased D2 receptor density in the basal ganglia compared to healthy controls (Beaulieu and Gainetdinov, 2011; Seeman, 2013). Early evidence for the involvement of the mesolimbic DA pathway in particular came from clinical findings that used implanted electrodes to show increased activity in limbic regions of schizophrenia patients during episodes of psychosis (Heath, 2005). Further following this were findings that high dose AMPH treatment induced a state with similarities psychosis, and animal studies revealed AMPH, which stimulates DA release from presynaptic terminals, had uniquely strong effects on the nucleus accumbens (NAc). Further still, AMPH-induced stereotypy observed in rodents could be ameliorated with haloperidol infusion to the NAc (McCutcheon et al., 2019). Schizophrenia patients release more DA in response to AMPH treatment than controls, although presynaptic D2 receptors, involved in feedback inhibition, are normal (Seeman, 2013). Overall, there are various alterations in DA-ergic function observed in schizophrenia.

The DA hypothesis has some shortcomings. Of particular importance, several fundamental symptoms of schizophrenia are not improved by conventional treatments involving antagonism at the D2 receptor. Cognitive impairment is a hallmark of schizophrenia, as well as negative symptoms which include avolition, anhedonia, and social withdrawal are persistently non-responsive to antipsychotics (Moghaddam and Javitt, 2012). These points imply other pathological avenues may be relevant.

1.2.2 The glutamate hypothesis

The glutamate hypothesis of schizophrenia postulates that abnormalities in the glutamatergic system, particularly driven by NMDA (N-methyl-D-aspartate) receptor hypofunction, are the underlying cause of the disease. NMDA receptor hypofunction was first implicated in the pathology of schizophrenia symptoms with the synthesis of phencyclidine (PCP) and ketamine in the 1960s, and the observation that administration to humans produced an acute psychotic state (Moghaddam and Javitt, 2012). The state induced by NMDA receptor antagonists recapitulates the appearance of schizophrenia, including the positive, negative, and

cognitive symptoms. They also exacerbate these symptoms in patients with schizophrenia, while other hallucinogens such as LSD do not (Jentsch and Roth, 1999).

Glutamate is the primary excitatory neurotransmitter of the brain and acts through ionotropic and metabotropic receptors (Traynelis et al., 2010; Moghaddam and Javitt, 2012). α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors are membrane-bound, tetrameric, ionotropic glutamate receptors located on postsynaptic neurons and are permeable to cations (K^+ , Na^+ , Ca^{2+}). NMDA receptors are classically regarded for their Ca^{2+} permeability which is 10-fold greater than Na^+ (Traynelis et al., 2010; Vyklicky et al., 2014). Upon activation and opening of the pore, Na^+ and Ca^{2+} travel along electrochemical gradients into the negatively charged intracellular environment to increase the electrical potential of the membrane and initiate depolarization, bringing the cell toward the threshold for firing an action potential; in the case of Ca^{2+} , intracellular signalling. In contrast, the opening of K^+ permeable channels result in efflux to hyperpolarize the cell. AMPA receptors are fast depolarizing channels that generate EPSCs to remove the voltage-gated Mg^{2+} block from NMDA receptors. In addition to the initial AMPA receptor-induced depolarization, NMDA receptors require simultaneous binding of glycine to the GluN1 subunit and glutamate to the GluN2 subunit for activation. Other related amino acids and molecules have agonist activity at NMDA receptors, such as enantiomers of serine at the glycine binding site and aspartate at the glutamate binding site and these also contribute to NMDA receptor signalling (Traynelis et al., 2010). NMDA receptor signalling forms a vital aspect of excitatory tone throughout the brain.

Accompanying the pharmacological evidence that NMDA receptor hypofunction could induce psychiatric symptoms in healthy human participants, additional evidence suggests altered glutamatergic transmission in schizophrenia. Cerebral spinal fluid (CSF) concentrations of glutamate in schizophrenia patients do not differ from controls, but assessments of post-mortem tissue from schizophrenic patients reveal deficient levels of NR1 subunit RNA and protein in the hippocampus, and animal models with genetic knockdown of NMDA receptors display numerous behaviour abnormalities (Kotecha and Macdonald, 2002; Moghaddam and Javitt, 2012). Several genetic polymorphisms for genes that code NMDA receptor subunits are associated with developing schizophrenia, and patients have altered regulation of glycine and D-serine levels, both of which modulate NMDA receptor activation (Moghaddam and Javitt, 2012).

1.2.2.1 MK-801 and other NMDA receptor antagonists

Compounds commonly used to pharmacologically induce NMDA receptor hypofunction in research settings include non-competitive antagonists MK-801 (dizocilpine), PCP (PCP), and ketamine. These are open channel blockers and ‘trapping’ blockers because they require the channel pore to be open in order to access the PCP-binding site, and they can become trapped within the channel (Traynelis et al., 2010). The PCP-binding site within the pore is distinct from the classic glutamate and glycine binding sites, but antagonists at these sites also induce the appearance of psychosis-like symptoms (Moghaddam and Javitt, 2012).

The consequences of systemic administration of MK-801 and similar compounds is generally referred to as NMDA receptor hypofunction, which contributes to the dysregulation of glutamatergic transmission through several proposed mechanisms. Interneurons that synapse onto cortical glutamatergic cells have a lower threshold for firing action potentials, resulting in greater basal depolarization and increased probability that voltage-gated NMDA receptor channels will be open, facilitating the binding of open channel blockers on these cells. The preferential block of NMDA receptors on interneurons results in disinhibition which may enhance glutamate release, destabilize cortical circuits, and prevent the coordination of cellular assemblies (Homayoun and Moghaddam, 2007; Moghaddam and Javitt, 2012). In addition, the blockade of NMDA receptors results in increased glutamate availability for binding other receptors and increased cellular activation in general. Overall, decreased binding at NMDA receptors can result in dysregulation of glutamate signalling in the brain by both reducing excitation of interneurons and increased AMPA receptor binding (Homayoun and Moghaddam, 2007; Moghaddam and Javitt, 2012).

In addition, there are links between NMDA receptor hypofunction and altered DA transmission (Laruelle, 2014). A recent meta-analysis of ketamine administration studies in rodents concluded that acute administration of ketamine results in elevated DA release in the cortex, striatum, and NAc, as well as increased DA neuron firing in the ventral tegmental area (Kokkinou et al., 2018). This is driven by the previously discussed disinhibition of glutamatergic signalling, including increased excitation of pyramidal cells and subsequent binding of AMPA receptors on DA-ergic neurons (Kokkinou et al., 2018). The levels of DA release in regions such

as the PFC and NAc is similar between ketamine, MK-801, and PCP (Kokkinou et al., 2018). In general, NMDA receptor antagonists are able to simulate a state of psychosis when administered acutely, and this can be tied to both main hypotheses of the underlying dysfunction in schizophrenia by affecting glutamatergic and DAergic transmission.

Figure 1.1: Schematic of the influence of NMDA receptor hypofunction on DA release.

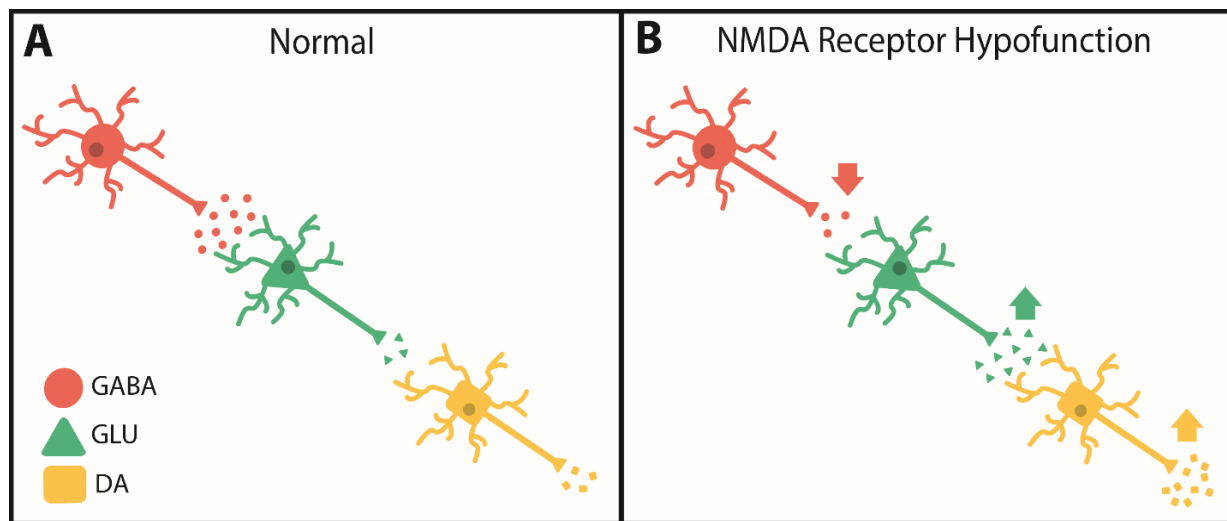


Figure 1.1: [A] A simple circuit of GABAergic, glutamatergic, and DAergic neurons to illustrate influences on DA release. [B] NMDA receptor hypofunction, as induced by MK-801 and similar drugs, primarily affects GABAergic neurons to result in reduced GABA release, disinhibition of the glutamatergic neuron, followed by increased glutamate release and influence on the DA-ergic neuron to induce greater DA release. The schematic is original, with the design influenced by Kokkinou and colleagues (2018).

1.2.3 Management of psychiatric illness

1.2.3.1 Conventional antipsychotics

Antipsychotic medications are the class of compounds used in the management of schizophrenia, schizophrenia spectrum disorders (SSDs), and other related illnesses. These compounds are recognized as either typical or first-generation antipsychotics (FGA), and atypical or second-generation antipsychotics (SGA) (Lally and MacCabe, 2015). FGAs are united by their shared pharmacological profile of D2 antagonism with greater receptor affinity than DA itself, and typically higher risk of adverse events than SGAs. The adverse events associated with FGAs in particular are severe, with a high risk for extrapyramidal side effects (EPSEs) including tardive dyskinesia, parkinsonism, akathisia, and acute dystonias. The primary pharmacological targets of SGAs are potent antagonism at the serotonin 5HT_{2a} receptor, and relatively weak antagonism at the D2 receptor. Compared to FGAs and DA itself, SGAs have lower binding affinity and more rapid dissociation from the D2 receptor. The transient binding but high receptor occupancy of SGAs was believed to carry a lower risk for side effects and greater efficacy in reducing symptoms, including the negative and cognitive symptoms which FGAs did not improve (Seeman, 2002; Meltzer et al., 2003; Lally and MacCabe, 2015). Several studies have now failed to find a difference between FGAs and SGAs efficacy, and while fewer EPSEs occur with SGAs, more systemic metabolic adverse events, such as weight gain, are seen. Ultimately, changes in quality of life and treatment discontinuation rates are similar between the two groups (as high as 75% within 2 years of hospital discharge; Lieberman et al., 2005; Jones et al., 2006; Lally and MacCabe, 2015).

Antipsychotic treatment is more effective than placebo, with 28% of schizophrenia patients experiencing symptom relapse within a year, compared to 68% who received placebo. 30% of patients with schizophrenia do not respond to either FGAs or SGAs and are classified as having treatment resistant schizophrenia (TRS) for which the only available and effective medication is clozapine. Clozapine is beneficial for up to 70% of TRS patients within a year of treatment, yet its use is relatively restricted due to the risk of life-threatening agranulocytosis and other adverse events (Lally and MacCabe, 2015). At present, the antipsychotic pharmacotherapies available do not fully meet the needs of patients, and no true innovation has occurred since the introduction of clozapine in the 1950s. Of the 3 symptom domains, only the

positive symptoms are reliably improved by treatment. Negative symptoms and cognitive impairment are core features of schizophrenia and remain unaffected by current treatment options. In addition, serious adverse events are common, as is treatment discontinuation (Young et al., 2009; Moghaddam and Javitt, 2012; Lally and MacCabe, 2015). There remains a need for innovative approaches to the pharmacological management of schizophrenia and related psychiatric illnesses (Lally and MacCabe, 2015).

Due to the increasing interest in the glutamate hypothesis, other novel drug targets are under exploration as antipsychotics. Despite indications that NMDA receptor hypofunction is implicated in the pathology of schizophrenia, targeting the NMDA receptor directly is a poor treatment strategy due to the harmful effects of indiscriminately enhanced NMDA activity. Sustained activation of NMDA receptors is neurotoxic, and the highly dynamic glutamate synapse responds rapidly to enhanced signalling with glutamate reuptake (Moghaddam and Javitt, 2012). Other strategies show greater promise by targeting glutamatergic transmission indirectly, such as allosteric modulation of presynaptic metabotropic glutamate receptors, are a current focus of innovation (Moghaddam and Javitt, 2012). Another approach continues to consider the DA hypothesis with novel manipulations that take advantage of the dichotomous effects of D1 and D2 receptors.

1.2.3.2 Govadine

There is a growing interest in a family of compounds derived from traditional Chinese medicine called tetrahydropyprotoberberines for putative efficacy as antipsychotics. One such synthetic compound is govadine (GOV; Lapish et al., 2012, 2014). GOV exists as two enantiomers with distinct, but complementary effects. Early preclinical studies in rats have begun to characterize the behaviour effects of GOV as a racemic mixture and as separate *d*- and *l*-enantiomers with evidence supporting benefits for all 3 symptom domains of schizophrenia (Lapish et al., 2012, 2014). Both enantiomers have high affinity for D1 DA receptors and increase DA efflux in the medial prefrontal cortex (mPFC), but *l*-GOV also has a high affinity for D2 receptors and increases DA efflux in the NAc as well (Lapish et al., 2012). *l*-GOV appears to act like a typical antipsychotic in that it shares several well-established characteristics of D2 antagonists, such as impairment of conditioned avoidance responding, reversal of AMPH-

induced hyperlocomotion and induces catalepsy. *d*-GOV displays cognitive-enhancing properties demonstrated by improving performance in the radial arm maze, blocking acquisition and reinstatement of conditioned place preference (to food and AMPH reward), as well as facilitates CPP extinction. Both enantiomers reverse AMPH-induced disruptions in latent inhibition, and social interaction deficits in the neonatal ventral hippocampal lesion model (Lapish et al., 2012, 2014). Interestingly, *l*-GOV has also shown efficacy in reversing MK-801 induced deficits in visual-spatial memory (Lins et al., 2015). The promising evidence for the potential of *d*- and *l*-GOV in managing the positive, negative, and cognitive symptoms of schizophrenia justify further investigation of these compounds.

1.3 Inflammation in pregnancy and offspring psychiatric outcomes

Psychiatric illnesses are complex and appear to occur as a result of combined genetic and environmental influences. The genetic contribution to schizophrenia etiology has long been evident due to the observable occurrence of increased risk in individuals with a diagnosed relative compared to those with no family history of schizophrenia. In identical twins, if one receives a diagnosis of schizophrenia, the other twin has an approximately 50% chance of also developing the disease. Despite the high heritability of schizophrenia, more recent genetics studies have not found a strong link to any single gene or polymorphism, and rather many weak associations have been detected. Further, the degree of heritability is not high enough to infer a purely genetic etiology, and exposure to environmental factors must also be considered (Insel, 2010). Gestational adversity encompasses a variety of insults that include maternal inflammation, maternal malnutrition, gestational diabetes, and neonatal hypoxia (Grissom et al., 2014; Brown and Meyer, 2018). This thesis includes studies of the impact of maternal inflammation on long-term psychiatric behaviour profiles of the offspring.

1.3.1 Early epidemiological evidence

Epidemiological studies demonstrate links between gestational and neonatal adverse events and elevated risks for the later development of schizophrenia in the offspring. These associations arose following observation of increased incidence of schizophrenia in the adult offspring of mothers from regions that experienced famine or widespread viral infections during

their pregnancies. Notable consideration has been given to the findings from a Finnish birth cohort. This study linked individuals in the second trimester of development during an influenza pandemic of 1957 with increased likelihood of receiving treatment at a psychiatric hospital with a diagnosis of schizophrenia by the age of 26 (Mednick, 1988; Brown, 2006). This initial study had several key limitations, such as lacking direct evidence that these individuals' mothers were infected, and gestational age during the epidemic was presumed based on birthdate with no data on preterm births; however, the study garnered attention and formed the basis for additional research (Brown, 2006).

1.3.2 Clinical prospective birth cohort studies

The association presented by Mednick and colleagues (1988) was eventually corroborated with the analysis of a richer dataset that included maternal serum collected throughout pregnancy, enabling confirmation of infection via the presence of antibodies to common viruses at the time, along with known gestational timing (Brown et al., 2004a). First trimester influenza exposure was associated with a 7-fold increase in risk of offspring developing an SSD, while broader definitions showed infection in the first half of pregnancy had a 3-fold increase in risk (Brown et al., 2004a).

Further studies continued to assess outcomes in prospective research cohorts. A notable analysis of long-term prospective data by Ellman and colleagues (2009) involved analysis of blood samples collected at birth, followed by various physical, cognitive, and psychological evaluations from birth to age 7. Maternal exposure to influenza infection in utero was serologically confirmed by the presence of antibodies for seasonal influenza viruses, and perinatal exposure to influenza infection was associated with lower scores on tests of cognitive performance in 7-year-old offspring that eventually received a psychiatric diagnosis compared to those that were not exposed (Ellman et al., 2009).

Autism spectrum disorders (ASD) have also been linked to maternal inflammation in pregnancy. Similar to the earlier studies that looked for associations with schizophrenia prevalence, there are mixed findings, although a review determined finding an association between infection in pregnancy and the occurrence of ASD depends on whether the studies included analysis of multiple covariates (Gardener et al., 2009). A more recent meta-analysis

assessed the relationship between maternal infection and offspring autism risk, with numerous confounding variables taken into account. Jiang and colleagues (2016) found that maternal infection is associated with a 12% increase in risk, although they acknowledged several limitations, including a lack of high-quality studies to include in their analysis. The final conclusion of the meta-analysis is conservative, stating that maternal infection may be associated with ASD, but notes given the prevalence of infection in pregnant women even a modest increase in risk could have substantial implications in disease prevalence (Jiang et al., 2016).

1.3.3 The cytokine hypothesis

In addition to the relationship between maternal influenza in pregnancy and SSD, infection via other pathogens (viral upper respiratory infections, herpes simplex virus-2 (HSV-2), measles, mumps, rubella, toxoplasma gondii) in pregnancy have been explored and linked to psychiatric illness risk in the offspring. Most of the risk-associated pathogens, including influenza, are not teratogenic (although there are exceptions such as rubella and toxoplasma gondii) which suggests there is an indirect, common mechanism for inflammation to impact fetal neurodevelopment. Cytokines are a candidate for this indirect mechanism (Brown, 2006).

Inflammatory events such as infection by a pathogen initiate a complex cascade of events to stimulate the release of immune proteins known as cytokines. Cytokines, a large family of proteins, glycoproteins, and peptides involved in signalling immune cells for recruitment and trafficking, are released mainly by peripheral immunocompetent cells during an immune response, but they are also produced by neurons and glia (Na et al., 2014). Although cytokines are most well known for their signalling roles in initiating an immune response, they also have roles in development, and maternal peripheral cytokines can cross the placenta and enter the developing CNS of the fetus where they have diverse influences (Deverman and Patterson, 2009). In very early development, a family of cytokines from the transforming growth factor beta (TGF β) family influence induction of the neuroepithelium, the source of neuroepithelial cells which give rise to neurons, astrocytes and oligodendrocytes. Inhibition of this signalling in mice results in a lack of forebrain development (Bachiller et al., 2000). Cytokines have also been implicated in the conversion of embryonic stem cells to neural stem cells in vitro. Once the cells of the neuroepithelium convert to radial glial cells, the gp130 family of cytokines encourage self-

renewal to maintain the RGC pool. TGF β signalling plays a role in the differentiation of tyrosine hydroxylase positive (TH⁺) DAergic neurons in the ventral midbrain by promoting conversion to this phenotype (Roussa et al., 2006; Deverman and Patterson, 2009). These findings illustrate that cytokines are implicated in neurodevelopmental processes, and altered cytokine signalling during critical time periods can influence neurodevelopment.

Chemokines are a subgroup of cytokines known to impact neurodevelopment. Within the immune system, chemokines use potent cellular attracting properties to recruit immune mediators to the site of an inflammatory insult. Chemokine receptors are constitutively expressed in CNS cells with roles in neurodevelopment, including directing neuronal migration, differentiation, and axonal pathfinding (Deverman and Patterson, 2009). The developmental roles of cytokine receptor CXCR4 and ligand SDF-1 has been amenable to investigation due to monogamous binding, and genetic knockout models display various developmental abnormalities in the cerebellum and dentate gyrus of the hippocampus. Other chemokine and chemokine receptor interactions are widely polygamous, complex, and dynamic, and understanding of their roles in development are still unfolding (Deverman and Patterson, 2009). While the mechanisms by which cytokines regulate and influence neurodevelopment are not fully known, heightened levels of inflammatory mediators, such as cytokines, during pregnancy may be responsible for long-term effects on the embryo that result in increased risk for the later development of psychiatric illness (Bergdolt and Dunaevsky, 2019).

Evidence that cytokines mediate the effects of infection during pregnancy on the eventual emergence of psychopathology in the offspring came from more prospective clinical studies that collected maternal blood samples during pregnancy for long-term follow-up. One study found a significant association between second/third trimester levels of IL-8, a chemokine, in pregnant women and their offspring's risk for schizophrenia, where levels of IL-8 were approximately twice as high in pregnancies where offspring eventually developed schizophrenia. This association was not seen for the other cytokines measured; tumor necrosis factor- α (TNF- α), IL-1 β , or IL-6 (Brown et al., 2004b). Another study used magnetic resonance imaging of the offspring as adults to determine the volumes of ventricular regions, and cortical, limbic, and subcortical structures. Among those offspring that developed SSDs, higher levels of interleukin-8 (IL-8) in maternal serum were associated with increased ventricular CSF volume, decreased

left entorhinal cortex volume, and decreased right posterior cingulate volume. These data were the first to demonstrate a relationship between measured maternal cytokine elevations and the neuroanatomical abnormalities in offspring that developed SSD (Ellman et al., 2010). Another study found significantly higher levels of cytokine TNF- α , but not IL-1 β , IL-2, IL-6, or IL-8, in late pregnancy in cases that eventually developed schizophrenia (Buka et al., 2001). Maternal serum cytokine elevations have been related to childhood psychiatric symptoms, with higher IL-8 levels in the first trimester associated with greater externalizing symptoms in the offspring, and higher IL-1ra (receptor antagonist) levels in the second trimester associated with greater internalizing symptoms in female offspring (Mac Giollabhui et al., 2019). Further research has associated elevated baseline cytokines in pregnancy with offspring outcomes, even in the absence of overt maternal illness. Working memory impairments and negative emotional affect were retrospectively linked to elevated IL-6 in maternal systemic circulation in children aged 2 years and 6 months respectively, and maternal IL-6 levels predict brain connectivity in newborns (Gustafsson et al., 2018; Rudolph et al., 2018).

In addition to studies of inflammation related to pregnancy and development, there is evidence of general immune disruption in patients with schizophrenia. Subjects with schizophrenia, and those at high risk of psychosis, have an altered profile of immune proteins in CSF indicative of higher baseline inflammation compared to healthy controls (Hayes et al., 2014). Long-term changes in baseline inflammatory mediators are also seen in animal models (Garay et al., 2013).

1.4 Animal models

1.4.1 Modelling psychiatric illness

The complex and heterogeneous nature of psychiatric illness challenges clinicians and researchers to categorize, diagnose, and develop animal models. The Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5) is the gold standard for the clinical diagnostic criteria of psychiatric illnesses. While the DSM-5 is a valuable resource for clinicians, the criteria are limited in their ability to inform preclinical research. The description of SSDs are illustrative of these challenges. Schizophrenia and related disorders are characterized by the presence of hallucinations, delusions, disorganized thoughts and speech, and behaviour often described as

inappropriate or bizarre. Distinct diagnoses include schizophrenia, schizoaffective disorder, delusional disorder, brief psychotic disorder, schizophreniform disorder, and substance-induced psychotic disorder (Beers, 2006). These conditions have a high degree of similarity and are often distinguished by the specific cluster of symptoms present in an individual, the duration or severity of symptom presentation (< 1 month, 1-6 months, or > 6months), or whether the symptoms cannot be better described with any other diagnosis. For example, the presence of non-bizarre delusions in the absence of other symptoms is the distinguishing characteristic that separates delusional disorder from schizophrenia. In addition, the DSM-5 diagnostic criteria require only a subset of criteria be met to form a diagnosis. This means two patients with no overlapping symptoms can receive the same diagnosis, complicating the process of accurately replicating disease features in any single model. This is further exacerbated as symptoms may overlap between distinct psychiatric diagnoses, such as major depressive disorder, which exhibits shared SSD symptoms including social withdrawal, avolition, and anhedonia (Nestler and Hyman, 2010). Despite the challenges of modelling psychiatric illness, several models are routinely used including acute pharmacological manipulations of relevant neurotransmitter systems, such as DA and glutamate, or early life insult to ‘prime’ for aberrant neurodevelopment, and these have led to valuable contributions to the understanding of such diseases.

1.4.2 Validity in models

Model systems are expected to demonstrate several levels validity for their use in research. Face validity refers to the ability of a model system to represent or resemble a disease or aspect of a disease. This can include key features such as biochemical, anatomical, or behavioural abnormalities present in disease relative to the control, or healthy condition (Nestler and Hyman, 2010). A key challenge in achieving face validity in psychiatric illness is the lack of biomarkers that could be objectively measured in a model, as well as the difficulty of fully representing the many factors present in complex, human psychiatric illness (Nestler and Hyman, 2010).

Construct validity refers to the manufacture or development of a model with respect to disease etiology or underlying disease pathology. Etiological validity requires the origins of a disease and the cause of abnormality in a model to be identical (Young et al., 2009). This can be

achieved using genetically modified animals where a known gene of interest is knocked down or overexpressed (Nestler and Hyman, 2010). Subjecting a laboratory animal to a known disease risk factor such as adolescent stress or maternal immune activation is another method to produce a model with some degree of etiological validity. In contrast, models produced by acute drug administration lack etiological validity for neurodevelopmental diseases but meet validity on other levels, such as construct validity by altering a neurotransmitter system known to be disrupted in disease. It is difficult to demonstrate full validity in models of psychiatric illness due to the complex gene-environment interactions that contribute to disease etiology (Nestler and Hyman, 2010).

Predictive or pharmacological validity is demonstrated when a known treatment for a disease, such as medication, is effective in correcting abnormalities reflective of disease symptoms in the model. Further complicating the application of predictive validity to animal models of psychiatric illness is the original use of rodent behaviour testing as a screening tool for drug discovery, rather than an indicator of modelled symptoms. Drug screens were developed based on the effects of known drugs on rodent behaviour with the hope that an experimental compound that induced similar behaviour effects would indicate its potential to treat psychiatric illness. However, the affected behaviour in rodents and the human disease were not necessarily intended to indicate disease symptoms or suggest that the behavioural abnormality and disease had similar underlying pathology, yet this is often how such screening tasks have been interpreted in the literature (Nestler and Hyman, 2010).

1.4.3 Acute pharmacological models

Several pharmacological agents induce a state that resembles acute psychosis when administered systemically and are commonly used to model psychiatric illness in research. An example of this is NMDA receptor antagonists, such as PCP, ketamine, and MK-801. In humans, acute administration of NMDA receptor antagonists induced all three symptoms domains seen in schizophrenia – positive, negative and cognitive; and this observation was instrumental in generating interest in the role of NMDA receptors in the disease (Jentsch and Roth, 1999). NMDA receptors have since been studied extensively in both human and animal subjects. Effects of acute systemic administration of NMDA receptor antagonists include increased extracellular

DA, glutamate and serotonin in animal models, and local infusion in the mPFC decreased extracellular γ -amino-butyric acid (GABA) in rats (Moghaddam et al., 1997; Jentsch and Roth, 1999; Greene, 2001; Bubenikova-Valesova et al., 2008). Acute MK-801 administration in rodents achieves face validity by producing abnormalities that resemble all three symptom domains as revealed by sophisticated behavioural testing, although it lacks the neurodevelopmental time course of disease progression (Adell et al., 2012). Behaviour effects of systemic NMDA receptor antagonists in animal studies include impaired working memory, PPI, social interaction, increased locomotion, and stereotypy (Jentsch and Roth, 1999). The finding that mRNA for parvalbumin (PV) is reduced in PV interneurons as soon as 24h following acute MK-801 treatment indicates that this acute pharmacological model can cause reduced patterns of GABAergic markers, one of the most robust findings seen in post-mortem schizophrenia cortical tissue (Romón et al., 2011). This supports that acute MK-801 administration mirrors relevant underlying disease factors and achieves a level of construct validity as a model of schizophrenia (Adell et al., 2012).

Enhanced DA receptor signalling with AMPH or APO is another common pharmacological approach to model schizophrenia. These models became popular following the observation that typical antipsychotic drugs block D2 receptors, and elevated midbrain DA concentrations are associated with the onset of psychotic episodes (Laruelle and Abi-Dargham, 1999). Hyperdopaminergia is commonly mimicked pharmacologically in rats by administration of APO, which has high affinity as an agonist at D1-like and D2-like receptors, but higher affinity at D2-like. It is important to note additional, direct effects of APO on serotonergic system with affinity for 5-HT1A and 5-HT2A receptors, and adrenergic system (Auffret et al., 2018). In both healthy human participants and patients with schizophrenia, elevated DA after AMPH treatment measured by positron emission tomography was associated with the severity of positive symptoms, with schizophrenic patients having increased sensitivity to the effects of AMPH (Laruelle and Abi-Dargham, 1999).

In animal models, heightened sensitivity to psychostimulants following enhanced DAergic activity, or MK-801-induced stereotypies taken to indicate positive symptoms of schizophrenia (see section 1.5, Behaviour Testing) can be reversed with haloperidol and other antipsychotics indicating predictive validity (Swerdlow et al., 1996). The acute MK-801-induced

negative symptoms do not respond to conventional treatments, but this is also predictive of effects in human patients because negative symptoms are not improved with current medications (Adell et al., 2012).

A notable drawback of acute pharmacological models is the lack of etiological validity. Schizophrenia follows a developmental time course, and this is lacking in acute drug models. Nonetheless, acute pharmacological models allow effects to be associated with the pharmacology of the compound used, are efficient means of inducing behaviour abnormalities consistent with psychiatric illness and have utility in screening for antipsychotic effects of potential therapeutic compounds (Bubenikova-Valesova et al., 2008; Nestler and Hyman, 2010). Acute MK-801 and APO are both used in Chapter 2 in order to assess the differential effects of the novel, putative antipsychotic GOV in the DAergic and glutamatergic dysfunction models of psychosis.

1.4.4 Neurodevelopmental models

As introduced in section 1.3, a body of evidence including epidemiology, neuropathology, and brain imaging studies resulted in the classification of schizophrenia as a neurodevelopmental disorder (Piontkewitz et al., 2012). It is generally accepted that an early life insult increases risk for psychosis disorders, and this has led to the development of rodent neurodevelopmental models. The standard protocol for developing the Maternal Immune Activation (MIA) model is to administer an immune stimulant to a pregnant rat or mouse (Piontkewitz et al., 2011a). This basic protocol can be modified by varying several parameters, including the type of immune stimulant used, the gestational timing of the insult, the dose of the immune stimulant, and route of administration of the maternal treatment. While the timing of the insult varies across studies and research groups, gestational day (GD) 15 is commonly used in rats. As reported by Zuckerman and colleagues (2003), limbic cortical neurons are proliferating and migrating during GD15, and earlier administration of the insult on GD13 resulted in unacceptably high rates of miscarriage. Administration on GD17 was also considered, but offspring behaviour effects were comparable to those seen in the GD15 treatment group. Kentner and colleagues (2019) review the many procedural variations of the MIA model and suggests a standardized list of modifiable factors for researchers to include for full transparency and to facilitate understanding of how these parameters influence outcomes in the offspring.

Polyinosinic:polycytidylic acid (polyI:C), a single stranded RNA molecule and viral mimetic with agonist activity at Toll-like receptor 3 (TLR3) is the most common compound used to induce MIA. Previous studies have confirmed that treatment with polyI:C during pregnancy increases peripheral pro-inflammatory cytokines including IL-1 β , IL-6, CXCL1 (the rodent homologue of IL-8), and TNF in the maternal circulation (Meyer et al., 2006; Hsiao and Patterson, 2011; Ballendine et al., 2015), imitating the effects of viral infection and inducing an acute inflammatory state. Lipopolysaccharide (LPS), a molecule found on the membrane of Gram-negative bacteria and which is also recognized by the innate immune system, is another common immune stimulant used in MIA (Arsenault et al., 2014; Wischhof et al., 2015). Other studies have used live pathogens, although these are less common due to many neuroscience laboratories lacking the expertise and access to the appropriate containment level standards required for this work (Kentner et al., 2019). Following maternal treatment, blood or tissue samples may be collected from the pregnant animals and embryos for further analysis of the effects of inflammation, or the offspring may be retained as research subjects after birth and followed throughout their lifespan.

MIA offspring display an array of behavioural, neuroanatomical, and functional brain changes reminiscent of schizophrenia (Meyer and Feldon, 2009; Piontkewitz et al., 2011a; Howland et al., 2012; Zhang et al., 2012; Meyer, 2014; Sangha et al., 2014; Ballendine et al., 2015; Lins et al., 2016). Structural brain changes documented in imaging studies emerge and diverge from control animals along a time frame that coincides with the onset of behaviour abnormalities at a post-pubertal developmental age and matches typical symptom emergence in humans (Zuckerman and Weiner, 2003; Ellman et al., 2010; Piontkewitz et al., 2011a). These structural changes include enlarged lateral ventricles and reduced hippocampal volume in polyI:C-exposed offspring at adulthood (PND 120) whereas no treatment differences were seen pre-puberty (PDN 35; Piontkewitz et al., 2012). The behavioural abnormalities seen in the rat offspring from polyI:C-exposed pregnancies include altered locomotor behaviour with and without the addition of psychotomimetic drug treatment. Some studies report reduced spontaneous locomotion and attenuated or exaggerated response to the hyperlocomotive effects of AMPH or MK-801 (Zuckerman et al., 2003; Zuckerman and Weiner, 2005; Bronson et al., 2011; Howland et al., 2012; Vorhees et al., 2012; Van den Eynde et al., 2014). There are also

mixed reports on the effects of MIA with several findings of impaired PPI and startle in the offspring, although this has not been reliably replicated (Fortier et al., 2004; Wolff and Bilkey, 2010, 2008; Dickerson et al., 2010, 2013, 2014; Howland et al., 2012; Klein et al., 2013; Ballendine et al., 2015; Hadar et al., 2015; Vorhees et al., 2015). Other behaviour abnormalities relevant to psychiatric illness include reduced social interaction, and impaired latent inhibition (Zuckerman and Weiner, 2003, 2005; Zuckerman et al., 2003; Lee and Green, 2016). Reduced working memory span (Murray et al., 2017), impaired associative memory (Ballendine et al., 2015), and mixed results regarding operant cognitive flexibility tasks (Zuckerman and Weiner, 2005; Zhang et al., 2012) have been revealed in MIA offspring using various learning and memory tasks. Hippocampal-dependent maze-based tasks of cognitive flexibility are impaired in MIA offspring and accompany enhanced paired-pulse facilitation and persistent long-term potentiation in the dentate gyrus, suggesting a link between altered hippocampal plasticity and impaired rule-switching (Savanthrapadian et al., 2013).

IL-6 is implicated in rodent models, as mice treated with IL-6 alone display behaviour abnormalities in tasks of latent inhibition and PPI, and blocking IL-6 alongside a synthetic inflammatory agent during pregnancy prevents the emergence of these abnormalities in the offspring, as does the use of IL-6 KO mice (Smith et al., 2007). The offspring of pregnant rats injected with human IL-6 display deficits in spatial learning and memory in the Morris Water Maze task, as well as neuron loss in the hippocampus (Samuelsson et al., 2006). Inflammation induced in pregnant rats using turpentine results in offspring with increased TH and DA in the NAc, as well as heightened sensitivity to the locomotor effects of AMPH, each of which is ameliorated when IL-6 antiserum is administered alongside the turpentine (Aguilar-Valles et al., 2012). There is some evidence from preclinical research that individual differences in maternal immune response are important in determining the degree of effects seen in the offspring. Pregnant rats that lose weight following administration of an immune stimulant have increased TNF- α , and their offspring show more severe deficits than those from dams that gained weight and did not have elevated TNF- α (Missault et al., 2014).

The rat MIA model of psychiatric illness meets several criteria for validity for schizophrenia. Etiological validity is achieved because heightened inflammation in pregnancy is a risk factor for the later development of schizophrenia in offspring (Brown and Meyer, 2018;

Kentner et al., 2019). Construct validity is met by the presence of underlying pathology such as an impaired DA system and abnormal mPFC-HPC coherence in MIA offspring (Dickerson et al., 2010; Luchicchi et al., 2016). However, there are ways in which MIA falls short of achieving true etiological validity. While inflammation in pregnancy is a risk factor for SSD, the true etiology of schizophrenia is likely multifaceted, and a single cause is unlikely for most cases. There are also known genetic risk factors not discussed here, although two-hit gene-environment, or stress-inflammation interaction models have been used by others (Giovanoli et al., 2013, 2014). To imply early-life exposure to inflammation only is the cause of psychiatric illness in humans would be oversimplified, therefore full etiological validity is not achieved. However, inflammatory insult is relevant to the development of psychiatric illness and the MIA model is valuable for discerning the influence of inflammation on offspring outcomes, and this relevance to the human disease contributes to construct validity. MIA has face validity in that all 3 schizophrenia symptom domains are represented in the model, as well as anatomical and biochemical abnormalities with a neurodevelopmental time course (Piontkewitz et al., 2011a; Luchicchi et al., 2016). The behaviour tasks that represent positive symptoms, such as AMPH-induced hyperlocomotion, respond to conventional antipsychotic drugs but cognitive impairments do not, an effect that mirrors the efficacy of these drugs in human patients and achieves predictive validity (Kentner et al., 2019). The final indicator of predictive validity is whether putative antipsychotic treatments with efficacy in improving negative and cognitive behaviour abnormalities in the MIA model translate to symptom improvement in human patients. MIA was used to model psychiatric illness in chapters 3 and 4 in order to determine if individual maternal cytokines levels are related to the severity of offspring psychiatric phenotype as analyzed with behavioural testing.

1.5 Rodent behaviour testing

1.5.1 Introduction to behaviour assays

Behaviour phenotyping is necessary in the study of complex psychiatric illness and neurodevelopment disorders (Silverman and Ellegood, 2018). Many disease models rely on the presence of disease-relevant biomarkers for validation, but clear biological indicators are lacking from many psychopathologies, and diagnoses are dependent on analysis of patient behaviour.

When improving functional outcomes in a patient population with behaviour alterations is of interest, animal models of such diseases should display relevant behaviours (face validity; further discussion in section 1.4, Animal models). The goal of translating preclinical animal research to inform the treatment of patient populations depends on the relevance of the assays used, and in the case of psychiatric illness, behaviour outcomes are paramount.

In addition to utility in the validation of disease models, behaviour assays have been used to screen compounds for potential therapeutic efficacy. In the case of antipsychotic compounds, direct observation of antipsychotic effects in a rodent model is challenging due to the complex nature of the clinical presentation of psychosis. The knowledge that D2 receptor antagonism was a common feature among first generation antipsychotics allows researchers to look for other behavioural evidence of antagonist activity at this pharmacological target, such as of catalepsy and Parkinsonian-like effects, to indicate potential efficacy as an antipsychotic (Nestler and Hyman, 2010).

Careful design of a rodent task battery is necessary for specific interpretation of behaviour effects. For example, if a test relies on the ability of an investigator to discern a rat's inclination to explore a novel environment as an indicator of an anxiety-like phenotype, the rat must not have a deficit in motor function, which is a known effect of typical antipsychotics. In this case, a basic assessment of locomotor activity is necessary to ensure the appropriate conclusions are drawn (Young et al., 2009). Similar to animal models, behaviour tasks can be assessed based on validity (section 1.4.2). In a behaviour task, construct validity, or determining whether a task measures what it is intended to measure is critical, but also incredibly challenging to achieve with certainty. Etiological validity is present when the biological phenomenon that underlies the effect is the same in the human and model. Predictive validity in the context of a behaviour test depends on the ability of a pharmacological agent effective in treating a human symptom also reversing the deficit or abnormality observed in the task (Young et al., 2009).

The behaviour tasks selected for the experiments outlined in data chapters 2 - 4 were intended to facilitate observation of the three symptom clusters of schizophrenia – positive symptoms, negative symptoms, and cognitive impairment.

1.5.2 Positive symptoms

The positive symptoms of schizophrenia refer to hallucinations and delusions, often described as characteristics that are present in schizophrenia that are not observed in a typical or healthy mental state (Nestler and Hyman, 2010). These symptoms also respond well to treatment with antipsychotic medication. Evaluating psychosis in rodents is often considered controversial due to the challenge of defining rodent behaviours consistent with hallucinations or delusions where the symptomatic behaviour may be uniquely human in nature. To circumvent this challenge, modeling the positive symptoms has relied on linking human symptoms and rodent behaviours that correlate with similar neurobiological features. Positive symptoms are believed to reflect abnormal and elevated DA levels, particularly in presynaptic subcortical DAergic neurons, and indeed several methodologies reveal evidence of this hyperDA-ergic state in schizophrenia. Specifically, increased DA synthesis in the striatum of schizophrenia patients is indicated by accumulation of [^{18}F]DOPA (Laruelle, 2014b). Schizophrenia patients also show greater DA release in response to AMPH than controls, and acute psychosis co-occurs with elevations in DA (Nestler and Hyman, 2010). In rodents, heightened locomotor activity in an open field is used as a proxy for psychosis as locomotion correlates with increased DA transmission, particularly in the ventral striatum (Meyer and Feldon, 2009). This has been compared to the elevated striatal DA levels seen in schizophrenia patients using single photon and positron emission tomography (Kegeles et al., 2010; Nestler and Hyman, 2010).

In addition to the relationship with DA levels, locomotor activity in rodents can be modified by pharmacological agents that manipulate neurotransmitter systems known to be disrupted in schizophrenia. DA is again a notable example which can be altered through agents such as AMPH and APO to enhance locomotion in rodents (Meyer and Feldon, 2009; Nestler and Hyman, 2010). These locomotor effects are blocked by typical antipsychotic compounds with D2 receptor antagonist activity, indicating predictive validity for this test (Nestler and Hyman, 2010). Glutamatergic manipulations are also relevant and NMDA receptor antagonists such as MK-801, PCP, and subanesthetic doses of ketamine are often used to induce a hyperlocomotive state, although NMDA receptor antagonism results in downstream elevations in striatal DA release as well (Figure 1.1; Meyer and Feldon, 2009; Moghaddam and Javitt, 2012; Kokkinou et al., 2018). The effects of glutamatergic manipulations in locomotor activity are

demonstrated to be variable across models of psychiatric illness and both hyper- and hypolocomotion have been reported. These effects have been attributed to the presence of NMDA receptor hypofunction in some models, or elevations in DA release downstream of the NMDA receptor to attempt to explain hypo- and hyperlocomotion, respectively (Zuckerman and Weiner, 2005; Giovanoli et al., 2013; Missault et al., 2014; Vorhees et al., 2015). MK-801 induced hyperlocomotion was used to detect positive symptom/psychosis-like behaviour in rats throughout this thesis (chapters 3 and 4).

1.5.3 Negative symptoms

The negative symptoms of schizophrenia are deficits in normal functions, including impoverished speech, social withdrawal, and avolition. Negative symptoms do not respond well to current treatment options and symptom origins are believed to be heterogeneous (Nestler and Hyman, 2010; Lee and Green, 2016). Social withdrawal or isolation is a negative symptom common in patients with schizophrenia (Lee and Green, 2016). Deficits in social behaviour are also a defining feature of autism, including impaired facial recognition (Silverman et al., 2010; Kennedy and Adolphs, 2012).

The task used to assess social behaviour in this thesis was the three-chambered Sociability Task and is described in detail in Chapters 3 and 4. In brief, a rat is allowed to explore a rectangular area divided into left, centre, and right compartments with the freedom to move between them. Either the left or right chamber contained a round cage that held a stranger rat, while the opposite side contained an identical but empty cage. Control rats show preference for exploring the cage containing a stranger and time spent in exploration of each cage can be compared across treatment groups. There are several task variations to assess social behaviour with one of the most common being the Social Interaction test in which 2 stranger rats are placed in an arena together. This is distinct from the three chambered task because the rats are free to approach and contact one another. The advantage of this is that different social behaviours can be clearly observed and scored by a trained investigator (grooming, sniffing, aggression, play) to produce a richer dataset (Silverman et al., 2010). The downside is that rats must be assessed in pairs and this makes it difficult to produce an individual measure of social behaviour which was necessary for the planned cytokine correlation analysis in Chapters 3 and 4.

Social behaviour appears to depend on multiple brain areas. Studies in humans with lesions reveal the temporal lobe is critical for processing faces, and the mPFC is involved in perceiving or understanding the beliefs or intentions of others. Amygdala damage also results in social deficits that are linked with recognizing emotions in others and modulating emotional outputs, although this may be an artifact of the broader role of the amygdala in emotional regulation and saliency, and not specific to sociability (Kennedy and Adolphs, 2012). In general, the neural regulation of social behaviour is diffusely represented throughout the brain and closely linked with other processes including cognition and emotional valence (Kennedy and Adolphs, 2012). Patients with schizophrenia do not prioritize social stimuli over non-social stimuli, do not attend to social cues as readily as non-social cues, and do not show improved memory for social stimuli. This was observed alongside reduced activation in the dorsomedial PFC (dmPFC) compared to healthy controls (Lee and Green, 2016).

Genetic mouse models of autism display impairments in various measures of social behaviour, including reciprocal social interactions, sociability, and preference for social novelty, as well as communication deficits indicated by reduced vocalizations (Silverman et al., 2010). Genetic and pharmacologically-induced NMDA receptor hypofunction also produce social deficits in mice. Furthermore, GABA_B agonist baclofen restores social interaction in NMDA receptor knockdown mice (NR1^{neo} ^{-/-}; Lee and Green, 2016). Taken together, social impairment seen in rodent models appears related to the loss of excitation/inhibition balance associated with NMDA receptor and GABAergic hypofunction and associated with the glutamate hypothesis of schizophrenia (Lee and Green, 2016).

1.5.4 Cognitive impairment

Cognitive impairment is a core symptom of schizophrenia, often present in the prodromal phase of the illness and persisting when psychosis is otherwise managed (Keefe and Fenton, 2007; Young et al., 2009; Nestler and Hyman, 2010). Seven affected cognitive domains have been identified: attention/vigilance; working memory; reasoning and problem solving; processing speed; visual learning and memory; verbal learning and memory; and social cognition (Nuechterlein et al., 2004; Young et al., 2009). Using rodent behaviour tests to examine symptoms of cognitive impairment is benefitted by the history of research into cognitive

processes in general. There are numerous well-studied rodent behaviour tasks sensitive to specific cognitive processes. In a well-controlled and designed behaviour test battery, deficits in performance can be taken to indicate cognitive impairment (Young et al., 2009).

Common cognitive tasks for rodents rely on innate and spontaneous rodent behaviour. An example is the spontaneous object recognition task (SOR) in which a rat is allowed to explore two identical copies of an object in an otherwise empty arena or Y-maze, and following a variable delay is reintroduced to the area with one copy of the original object alongside a novel object. Due to an innate and reliable preference for novelty, control rats spend a greater amount of time exploring the novel object and this is interpreted by an investigator to imply memory for the original object is intact. This task allows for various manipulations to further investigate mechanisms of memory and has been used extensively to understand aspects of cognition (Lyon et al., 2012; Warburton and Brown, 2015). Common variations include object-location memory, object-in-place memory and temporal order memory (Warburton and Brown, 2015).

The two variations of spontaneous exploration behaviour tasks seen in this thesis are Crossmodal Object Recognition Memory (CMOR) and Oddity Discrimination, or Odd Object Preference Task. The procedure for CMOR is described in detail in the subsequent data chapters 3 and 4. Briefly, the task occurs in a Y-maze and consists of three distinct trials; a tactile, visual, and CMOR, conducted in that order on separate consecutive days following two days of habituation to the test apparatus. Each trial is further divided into a sample and test phase, like the classic version of SOR. The sample phase presents two identical copies of an object in each arm of the maze. The test phase, one hour later, presents a third copy of the first object and a novel object. The tactile test occurs in red light conditions, which prevents the rats' visual observation of the objects. The visual test occurs in white light, but clear Plexiglas barriers inserted in front of the objects prevent tactile exploration. The CMOR phase has a tactile sample phase and a visual test phase. The objects used are unique to each trial (Winters and Reid, 2010; Cloke et al., 2015).

It is increasingly recognized that sensory integration is altered in various psychiatric conditions, including schizophrenia and autism. Some evidence for impaired multisensory integration in patients with schizophrenia and autism includes reduced susceptibility to the McGurk effect, a phenomenon where the presentation of incongruent audio and visual stimuli

produce the perception that of a single stimulus that combines the two conflicting stimuli. In addition, cognitive performance is enhanced in healthy participants when congruent information is acquired through multiple modalities, an effect that is dampened in patients with schizophrenia (Cloke et al., 2015). Lesion studies in rats have contributed to understanding the brain regions involved in the CMOR task, as well as the unimodal tactile and visual memory tests. The posterior parietal cortex (PPC) appears necessary for tactile memory performance while the perirhinal cortex (PRh) in the temporal lobe is necessary for visual recognition memory. Orbitofrontal cortex (OFC) lesions selectively impair CMOR while sparing performance of the visual and tactile unimodal tasks, consistent with the anatomical convergence of various sensory modalities in this region. This impairment is delay-dependent and not seen when the test phase immediately followed the sample phase, suggesting the role of the OFC is mnemonic and not necessary for the integration of visual and tactile modalities (Cloke et al., 2015). Further research has begun to assess the roles of other areas with anatomical convergence of sensory inputs. Two candidate regions are the claustrum and retrosplenial cortex which, when lesioned, selectively impair CMOR. Despite the well-known role of the hippocampus in memory, hippocampal lesions do not impair CMOR (Cloke et al., 2015). CMOR has been studied using MIA models and selective disruptions in the crossmodal phase are seen while tactile and visual memory remain intact (Ballendine et al., 2015; Paylor et al., 2018). Systemic MK-801 and ketamine administration impair CMOR, but not visual or tactile memory at short delays, indicating relevance to the glutamate hypothesis of schizophrenia (Cloke et al., 2016).

The procedure for oddity discrimination is described in detail in data chapters 3 and 4. Briefly, the task occurs in a square arena with Velcro in each of the 4 corners. After three days of arena habituation, the task occurs in a single phase. 3 identical objects, and one different or ‘odd’ object, are fixed in place to the Velcro and the rat is allowed to explore for 5 minutes. Control rats explore the odd object at a greater than chance level. Oddity discrimination is a relatively novel behaviour task, particularly the variation of the task used here, and little is known of the neural underpinnings of task performance. Like CMOR, sensory integration is believed to contribute to oddity discrimination task performance, but there is no separation of sensory modalities and no mnemonic component. The PRh has been implicated due to a well validated role in visual discrimination, and studies on variations of the oddity discrimination task provide

evidence for a role of PRh in perception, yet this remains controversial (Bussey and Saksida, 2005; Buckley and Gaffan, 2006; Suzuki, 2009). Despite the lack of data on this particular variant of oddity discrimination, the PRh has been implicated in object identification and discrimination, abilities on which this task likely depends (Murray and Richmond, 2001). Object recognition maps onto the cognitive domain of visual learning and memory, which is impaired in patients with schizophrenia and reduced temporal lobe volume is a common anatomical alteration seen in patients (Young et al., 2009; Ellman et al., 2010; Lyon et al., 2012).

The procedure for the operant set shifting and reversal task (OSST) is described in detail in Chapter 3. Briefly, the task is conducted in modular metal chambers where one wall is equipped with 2 stimulus lights (left and right), each located above a corresponding retractable lever. A food reward port was located between the levers. Rats are initially trained to press the levers in order to receive an appetitive reward, then to press the lever (left or right) the corresponds to a visual cue, i.e. the illuminated stimulus light. On a subsequent day, the rule changes (set shift) and the rats must select the lever opposite to their preferred side in order to receive a reward. On another subsequent day, the rule changes once more and the rats must select the opposite side as the previous day (reversal). The pairwise discrimination and reversal learning task (PD/RL) bears many similarities to OSST and is also described in detail in Chapter 3. PD/RL uses a touchscreen chamber and displays 2 visual stimuli, one is always rewarded and the other is never rewarded. Once the rat has reached a performance criterion the initially unrewarded stimulus becomes rewarded, and the initially rewarded stimulus becomes unrewarded, requiring the rat to switch behavioural response to continue receiving a food reward (reversal) and again is trained until a criterion is met.

Both OSST and PD/RL are tests of executive function that require cognitive flexibility, the ability to solve problems by responding to changing demands over time by shifting behaviour appropriately; a previous strategy is disengaged and the initial response is inhibited, while a new strategy learned and employed (Young et al., 2009). Set-shifting requires a participant to shift response from one stimulus dimension to a to a previously irrelevant dimension (extradimensional shift, EDS), while reversal learning requires a shift in response to another stimulus within the same dimension (intradimensional shift, IDS). The Wisconsin Card Sort Task (WCST) and Neuropsychological Assessment Battery – Mazes tasks demonstrated human

patients with schizophrenia display abnormalities in executive function, particularly taking more time to change strategy and making more errors by reverting to a previously correct strategy, known as perseveration, compared to controls (Waltz, 2017). Patients with frontal lobe damage are profoundly impaired at strategy shifting with a characteristic high rate of perseverative errors. The data from patients with schizophrenia are somewhat more variable than those of clinical lesion studies. While increased perseveration are observed, patients with schizophrenia also make more errors of other kinds, and impaired cognitive flexibility has come to be known as a hallmark of cognitive dysfunction in schizophrenia (Floresco et al., 2009; Waltz, 2017).

OSST and PD/RL are cognitive flexibility tasks adapted for automated testing of rodents using modular lever boxes or a computerized touchscreen chamber system, respectively. Both tasks involve learning an initial visual cue, then OSST includes an extradimensional shift (from visual cue to spatial cue), then an intradimensional shift (from spatial cue to the opposite spatial cue), while PD/RL only includes an intradimensional shift (from one visual stimulus to the other visual stimulus). There are three types of errors that can be made during OSST. The first are the previously described perseverative errors, defined as the reverting to selecting the previously rewarded choice, indicative of a failure to disengage a presently irrelevant strategy. Once a rat has begun to disengage from a previous strategy and is perseverating less than 25% of the time, selection of the previously relevant stimulus is then considered a regressive error, the presence of which indicates difficulty in novel strategy maintenance. Finally, a never reinforced error is the employment of a strategy that has never been rewarded and serves to represent the ability to abandon incorrect strategies (Floresco et al., 2009).

Rodent studies have been instrumental in delineating the neurological underpinnings of cognitive flexibility, building upon the early studies in human lesion patients. Lesion or inactivation of the mPFC, homologous to the primate dorsolateral PFC (dlPFC), impairs set-shifting and increases perseveration, indicating importance of that region for disengaging a previous strategy. Reversal learning, but not set-shifting, is impaired with lesion or inactivation of the OFC, revealing this region is involved in linking a stimulus to a rewarded outcome (Floresco et al., 2009). The striatum is a major input region of the mPFC and have also been implicated in behavioural flexibility, with the NAc core facilitating the adoption of novel strategies by suppressing ineffective responses and the NAc shell ignores irrelevant stimuli,

while the dorsal striatum enacts goal directed behaviour and may be involved in responding to changing rules (Floresco et al., 2009; Bissonette and Roesch, 2017). These regions have been implicated in psychiatric illness, particularly the circuits involved in the psychopathology of schizophrenia (Floresco et al., 2009). Altered cognitive flexibility has been demonstrated in rodent models of schizophrenia including MIA in OSST, as well as pharmacological models MK-801, PCP, AMPH, or APO in PD/RL and MK-801 and ketamine in OSST (Floresco et al., 2009; Talpos et al., 2012; Zhang et al., 2012).

1.5.5 Prepulse inhibition

Prepulse inhibition (PPI) requires a separate consideration as it does not represent a symptom of schizophrenia per se, yet it is highly relevant to characterising psychiatric phenotypes, and screening compounds for antipsychotic potential. PPI is the reduced response to a startling stimulus when closely preceded by a low-intensity stimulus and is a measure of sensorimotor gating, an inhibitory mechanism believed to regulate sensory input. PPI has the rare benefit using the same testing paradigm across multiple species including humans, often using auditory tones and subsequent startle reflex, but blink reflex (air pulses into the eye) and crossmodal paradigms have also been used. The neural circuitry regulating PPI involves limbic, cortical, striatal, and pontine brain regions (Swerdlow et al., 2001b).

Deficits in PPI are not specific, and are seen across various psychopathologies including schizophrenia, Alzheimer's disease, Huntington's disease, and Tourette's syndrome, but notably not impaired in bipolar disorder or major depressive disorder (Swerdlow et al., 2016b). PPI can be disrupted with administration of DA agonists AMPH or APO, and NMDA receptor antagonists such as MK-801, and the ability of a compound to reverse PPI deficits is predictive of antipsychotic activity (Swerdlow et al., 2006a). These effects reveal relevance to the neurotransmitter systems altered in schizophrenia as well as predictive validity. Further, typical antipsychotic compounds reverse PPI deficits when disrupted by DA agonists, but not NMDA receptor antagonists. Atypical antipsychotics have mixed results in their ability to ameliorate NMDA receptor antagonist-induced deficits, but there are reports of reversal by clozapine, chlorpromazine, olanzapine and quetiapine (Bakshi et al., 1994; Swerdlow et al., 1996, 1998; Chapter 2 includes further discussion of PPI and the effects of antipsychotic drugs). In the

context of schizophrenia, PPI is occasionally linked to positive symptoms due to the reliable effect of DA agonists in disrupting the typical inhibition of startle when followed by a prepulse. This is comparable to the use of locomotor activity as a positive symptom proxy due to its relationship to biological correlates such as cortico-striatal DA levels, despite lacking a direct resemblance to psychosis. PPI may also bear relevance to cognition, as sensorimotor gating has been considered a pre-cognitive process, necessary for sustained attention, sensory processing, and the prevention of cognitive fragmentation (Swerdlow et al., 2006a; Scholes and Martin-Iverson, 2009; Young et al., 2009). More recently however, this view has been challenged as a large meta-analysis was unable to correlate PPI with cognitive performance in human patients with schizophrenia, and PPI does not decrease with normal ageing, although it is associated with lower Global Assessment of Function scores and measures of thought disorder suggesting there is value in PPI deficits as an indicator of abnormal sensory processing (Swerdlow et al., 2006b; Young et al., 2009). Overall, behavioural assessment of PPI bears relevance to multiple domains of psychiatric illness, is easily tested in rodents, and has translational relevance to human disease with similar testing paradigms across species, and predictive validity for screening compounds for potential antipsychotic activity.

Despite many positive aspects of PPI for research, there are also a number of limitations. PPI is sensitive to species and strain effects, and the vast number of possible parameter manipulations results in a literature that is complex and difficult to generalize. In addition, despite the predictive validity of PPI in regard to antipsychotic efficacy, homology of the underlying circuitry cannot be assumed across species and this has been exemplified in comparing mice to rats (Swerdlow et al., 2001b, 2016b).

1.6 General hypotheses

The general hypotheses of this thesis are:

(A) ι -GOV, but not α -GOV, will reverse PPI impairments caused by APO and MK-801; (B) Serum concentrations of inflammatory proteins collected from pregnant dams will correlate with the severity of their offspring's behavioural phenotype relevant to psychopathology; (C) Male polyI:C offspring will display a greater degree of pathology than females.

1.7 Thesis objectives

- 1) Establish the ability of putative antipsychotics d_1 - and l_1 -GOV to attenuate PPI impairments due to DA receptor hyperfunction following acute APO treatment (Chapter 2).
- 2) Establish the ability of putative antipsychotics d_1 - and l_1 -GOV to attenuate PPI impairments due to NMDA receptor hypofunction following acute MK-801 treatment (Chapter 2).
- 3) Determine the long-term effects of inflammation in pregnancy on male offspring behaviour related to psychiatric illness, and whether phenotype severity is correlated with maternal serum concentrations of cytokines CXCL1 or IL-6 (Chapter 3).
- 4) Determine the long-term effects of inflammation in pregnancy on female offspring behaviour related to psychiatric illness, and whether phenotype severity is correlated with maternal serum concentrations of cytokines CXCL1 or IL-6 (Chapter 4).
- 5) Assess the male and female behaviour outcomes for sex effects (Chapter 4).

CHAPTER 2

DISSOCIABLE EFFECTS OF THE D- AND L- ENANTIOMERS OF GOVADINE ON THE DISRUPTION OF PREPULSE INHIBITION BY MK-801 AND APOMORPHINE IN MALE LONG-EVANS RATS¹

2.1 Abstract

Rationale The search for novel antipsychotic drugs to treat schizophrenia is driven by the poor treatment efficacy, serious side effects, and poor patient compliance of current medications. Recently, a class of compounds known as tetrahydroprotoberberines, which includes the compound d,l-govadine (d,l-GOV), have shown promise in preclinical rodent tests relevant to schizophrenia. To date, the effect of GOV on PPI, a test for sensorimotor gating commonly used to assess the effects of putative treatments for schizophrenia, has not been determined.

Objectives The objective of the present study was to determine the effects of each enantiomer of GOV (d- and l-GOV) on PPI alone and its disruption by the distinct pharmacological compounds apomorphine and MK-801.

Methods Male Long-Evans rats were treated systemically with d- or l-GOV and apomorphine or MK-801 prior to PPI. The PPI paradigm employed here included parametric manipulations of the prepulse intensity and the interval between the prepulse and pulse.

Results Acute MK-801 (0.15 mg/kg) significantly increased the startle response to startle pulses alone, while both MK-801 and APO (0.2 mg/kg) significantly increased reactivity to prepulse-alone trials. Both MK-801 and APO disrupted PPI. In addition, d-GOV alone significantly disrupted PPI in the APO experiment. Pretreatment with l-, but not d-, GOV (1.0 mg/kg) blocked

¹ **Lins BR**, Marks WN, Phillips AG, Howland JG (2017) Dissociable effects of the d- and l- enantiomers of govadine on the disruption of prepulse inhibition by MK-801 and apomorphine in male Long-Evans rats. *Psychopharmacology (Berl)* 234:1079–1091.

Lins and Marks were co-first authors who contributed equally to the manuscript; Phillips supplied govadine and edited the manuscript; Howland designed and supervised the experiments and co-wrote the manuscript. This manuscript is peer-reviewed and published.

the effect of APO and MK-801 on PPI. Treatment of rats with ι -GOV alone (0.3, 1.0, 3.0 mg/kg) also dose-dependently increased PPI.

Conclusions Given the high affinity of ι -GOV for DA D2 receptors, these results suggest that further testing of ι -GOV as an antipsychotic is warranted.

2.2 Introduction

Schizophrenia is a debilitating psychiatric disorder that affects approximately 1% of the global population. Current treatment options are not effective for all symptoms of the disorder and as a result, novel compounds with potential for use in schizophrenia are currently under development. One family of compounds known as tetrahydroprotoberberines (THPBs), derived from traditional Chinese medicine, have unique DA-related activity (Jin et al., 2002; Yang et al., 2007; Natesan et al., 2008; Lapish et al., 2012; Zhai et al., 2012). GOV is a synthetic THPB that exists as two enantiomers, d - and ι -GOV (d -GOV, ι -GOV), each with distinct pharmacological profiles (Lapish et al., 2012; Zhai et al., 2012). Affinity for D1 and D2S classes of DA receptors is comparable between d - and ι -GOV. However, a large difference in affinity for the D2L receptor exists between ι -GOV (165 nM) and d -GOV (1340 nM). Affinities for other receptor types that are comparable between the two enantiomers are low in relation to these different classes of DA receptors (Lapish et al., 2014). Studies using microdialysis determined ι -GOV increases DA efflux in both the prefrontal cortex (PFC) and NAc, whereas d -GOV only increases DA efflux in the PFC (Lapish et al., 2014). Previous research using rats suggests GOV may have the unique ability to improve the three major symptom categories seen in schizophrenia: positive, negative, and cognitive (Lapish et al., 2012, 2014). When examined separately, ι -GOV, but not d -GOV, blocks AMPH-induced hyperlocomotion, impairs conditioned avoidance responding, and induces catalepsy similarly to antipsychotic drugs with high affinity for the D2L receptor. In contrast, d -GOV displays primarily pro-cognitive effects by reducing errors on the delayed-spatial win-shift task and improving temporal order memory at a long delay. Both enantiomers successfully reversed social interaction deficits, a measure of negative symptoms, as well as AMPH-disrupted latent inhibition (Lapish et al., 2014). ι -GOV, but not d -GOV, reverses MK-801- induced impairments in a touch-screen visuo-spatial paired associates learning task (Lins et

al., 2015). These data encourage further investigation of GOV enantiomers as a putative treatment for schizophrenia.

PPI refers to the reduced motor response to a startling stimulus, such as an auditory tone or “pulse,” when the startling stimulus is preceded by another low-intensity sensory input in close temporal proximity (Koch and Schnitzler, 1997; Geyer et al., 2001; Yeomans et al., 2006). Therefore, PPI is a common measure of sensorimotor gating used in studies of humans or animals including rodents and has cross-species validity, face validity, ease of implementation, and reliability. The predictive validity of PPI is related to the finding that compounds which reverse drug-induced PPI disruptions in rodents very often have antipsychotic efficacy in humans (Swerdlow et al., 2006a, 2016b, 2016a). PPI is commonly measured with auditory pulses and prepulses, although other cross-modal paradigms have been developed. PPI is impaired in several psychiatric illnesses including schizophrenia (Braff et al., 1978; Braff and Geyer, 1990; Grillon et al., 1992), obsessive compulsive disorder (Swerdlow et al., 1993), Huntington’s disease (Swerdlow et al., 1995), and Tourette’s syndrome (Castellanos et al., 1996).

The neural circuitry regulating PPI includes an array of limbic, cortical, striatal, pallidal, and pontine brain areas collectively known as the “CSPP” circuitry (Swerdlow et al., 2001b, 2016b, 2016a). Multiple sequential and parallel connections including the temporal cortex, mPFC, ventral striatum, ventral pallidum, and pontine tegmentum converge at the nucleus reticularis pontis caudalis in order to regulate PPI. The complete anatomical circuitry of PPI is extensive and includes a network of additional brain regions (reviewed in Swerdlow et al., 2001b). PPI is disrupted following systemic administration of the DA agonists APO and AMPH (Geyer et al., 1987; Mansbach et al., 1988) and noncompetitive NMDA receptor antagonists such as MK-801 (dizocilpine) (Mansbach and Geyer, 1989, 1991; Al-Amin and Schwarzkopf, 1996; Bast et al., 2000). The effects of typical and atypical antipsychotic drugs on PPI have been studied extensively. Typical antipsychotics such as haloperidol, a D2 antagonist, effectively reverse deficits in PPI induced by acute pharmacological challenge with APO (Swerdlow and Geyer, 1993) but not MK-801 or PCP (Geyer et al., 1990; Keith et al., 1991; Johansson et al., 1994). In contrast, atypical antipsychotics reverse the impairments following either APO or MK-801 treatment, although some inconsistencies have been reported. APO-induced deficits are blocked by clozapine (Swerdlow and Geyer, 1993) whereas clozapine, quetiapine, and

olanzapine either improve (Bakshi et al., 1994; Bakshi and Geyer, 1995; Swerdlow et al., 1996, 1998; Zhang et al., 1997; Bubeníková et al., 2005) or do not affect acute MK-801- induced impairments in PPI (Bast et al., 2000). Zotepine and risperidone do not restore PPI following MK-801 treatment (Swerdlow et al., 1996; Varty et al., 1999; Bubeníková et al., 2005). Further, PPI disrupted by either MK-801 or PCP is resistant to reversal by specific antagonism of D1, D2, or 5-HT₂ receptors (Keith et al., 1991; Bakshi et al., 1994). When administered alone, olanzapine and clozapine also decrease PPI in some studies (Bubeníková et al., 2005) but not others (Depoortere et al., 1997). The distinctions in PPI disruption caused by APO or MK-801 and the different approaches needed to ameliorate them may be relevant to different schizophrenia patient populations, with those compounds that reverse effects of MK-801 having the prospect of antipsychotic efficacy for individuals resistant to current therapies (Al-Amin and Schwarzkopf, 1996).

Given the distinct effects of typical and atypical antipsychotic drugs on the disruption of PPI by APO and MK-801, we tested the effects of each enantiomer of GOV on these drug-induced disruptions of PPI. PPI can fluctuate depending on the interaction of drug treatment with specific PPI protocol parameters in both clinical populations and rodents (Duncan et al., 2001; Swerdlow et al., 2008, 2016b, 2016a; Howland et al., 2012; Ballentine et al., 2015; Chandna et al., 2015; Pinnock et al., 2015). Therefore, the PPI protocol employed a range of prepulse-pulse intervals (30, 50, 80, and 140 ms) and prepulse intensities (3, 6, and 12 dB). We hypothesized that *l*-GOV, but not *d*-GOV, would block the PPI impairments caused by APO, consistent with its strong DA D₂L receptor antagonist activity. The heterogeneity of antipsychotic drug effects on the MK-801-induced disruption of PPI in previous reports made it difficult to develop a clear a priori hypothesis regarding the effects of *d*- or *l*-GOV in this paradigm. We also measured the effects of all drugs on the startle response and prepulse-elicited reactivity (Yee and Feldon, 2009). Given the effectiveness of *l*-GOV in blocking the PPI impairments caused by APO and MK-801, we also conducted a dose-response experiment of the effects of *l*-GOV (0.3, 1.0, 3.0 mg/kg) alone on PPI.

2.3 Materials and methods

2.3.1 Animals

Adult male Long-Evans rats (Charles River Laboratories, Quebec, Canada) weighing 325–500 g throughout the course of testing were group housed (two per cage) in standard polypropylene cages in a temperature-controlled (21 °C) colony room on a 12:12 h light/dark cycle with food (Purina Rat Chow) and water available ad libitum. Experimental procedures were carried out during the light phase (lights on at 0700 hours). All experimental procedures were conducted in accordance with the Canadian Council on Animal Care and were approved by the University of Saskatchewan Animal Research Ethics Board.

2.3.2 Drug preparation

Apomorphine (0.2 mg/kg, Howland et al. 2004; Sigma, St. Louis, MO) was dissolved in 0.1% ascorbic acid in water. MK-801 (0.15 mg/kg; Lins et al. 2015; Abcam, Toronto, Ontario) was dissolved in water. *d*- and *l*-GOV were synthesized by the Sammis Lab (Department of Chemistry, University of British Columbia) and dissolved in a 1 mg/mL solution of 50% dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO) and 50% water. Each drug was administered at a volume of 1 mL/ kg bodyweight. Initially, we elected to use a single dose of *d*- and *l*-GOV based on previous findings reporting dose-response effects of GOV in several paradigms associated with symptoms of psychotic behaviour. In these cases, in the absence of drug effect at a dose of 1.0 mg/kg, doses up to 10 times greater also failed to yield effects (Lapish et al., 2014). A recent study by our group on the effects of each enantiomer of GOV in a visual-spatial learning and memory task showed *l*-GOV (1.0 but not 0.3 or 3.0 mg/kg) effectively reversed MK-801 induced impairments whereas *d*-GOV had no effect at doses up to 3.0 mg/kg (Lins et al., 2015). Based on these data, we chose to administer 1.0 mg/kg of each enantiomer in conjunction with APO and MK-801 to assess effects on PPI and startle reactivity. Positive effects of *l*-GOV (1.0 mg/kg) encouraged a separate dose-response experiment with *l*-GOV (0.3, 1.0, and 3.0 mg/kg) alone using the same PPI protocol. It should be noted that the use of a single dose of GOV with APO and MK-801 is a limitation for the study and a full dose-response characterization of the effects of *d*- and *l*-GOV on disrupted PPI would be valuable.

2.3.3 Behavioural testing

Rats were handled in small groups for 5 min/day at least three times before the first PPI session. The PPI testing procedure was conducted according to a previously published protocol (Howland et al., 2012; Ballendine et al., 2015). Two SR-Lab startle boxes (San Diego Instruments, San Diego, CA, USA) were used. Each testing session began with 5-min acclimatization during which a background noise (70 dB) was presented and remained constant for the entire testing period. After acclimatization, six pulse-alone trials (120 dB, 40 ms) were presented to obtain steady startle amplitude. Following the 6 pulse-alone trials, 84 trials of three types were presented in pseudorandom order: pulse-alone (6 trials; 120 dB, 40 ms); prepulse + pulse (72 trials; parameters described below); or no stimulus (6 trials). Prepulse + pulse trials began with a 20-ms prepulse of 3, 6, or 12 dB above background noise (70 dB). Four different prepulse—pulse intervals of 30, 50, 80, or 140 ms—were used between the onset of the prepulse and the onset of the 120-dB pulse. Six trials of each prepulse × prepulse-pulse interval combination were presented. Each testing session ended with another six pulse-alone trials (120 dB, 40 ms). The inter-trial interval varied from 3 to 14 s (average 7.5 s) in random order. After each session, the startle boxes were cleaned with 40% ethanol.

In experiment one, rats ($n = 20$) received six treatments in a counterbalanced, Latin square, within-subjects design: vehicle, d -GOV, l -GOV, APO, APO + d -GOV, and APO + l -GOV. GOV was administered 15 min prior to APO which was given immediately prior to PPI (Howland et al., 2004). In experiment two, a separate group of rats ($n = 19$) was tested similarly to experiment one using the following treatments: vehicle, d -GOV, l -GOV, MK-801, MK-801 + d -GOV, and MK-801 + l -GOV. GOV was administered immediately prior to MK-801, 15 min before starting PPI (Lapish et al., 2014; Lins et al., 2015). In experiment three, a third cohort of rats ($n=12$) was tested in the same PPI protocol using three doses of l -GOV (0.3, 1.0, and 3.0 mg/kg) as well as vehicle injections 15 min prior to PPI. All injections were administered via the subcutaneous (s.c.) route except for MK-801, which was administered intraperitoneally (i.p.). In all experiments, PPI sessions were conducted every 3–4 days until all treatments were complete. Repeated treatments with APO or MK-801 were administered a minimum of 6 days apart to reduce potential sensitization effects.

2.3.4 Statistical analyses

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 21 for Windows (IBM, Chicago, IL). Greenhouse-Geisser corrections were used for instances of sphericity violations (Mauchley's Test) for all repeated measures analysis of variance (ANOVA) with no adjustments otherwise. Post hoc analyses were performed using Tukey's test. Statistical significance for all comparisons was $p \leq 0.05$. PPI was calculated by averaging the startle amplitudes for each trial type, and the percent PPI for each prepulse intensity was calculated using the formula: $[100 - (100 \times \text{startle amplitude on prepulse} + \text{pulse trials}) / (\text{startle amplitude on pulse-alone trials})]$. PPI was observed for the 50-, 80-, and 140-ms interval whereas the 30-ms interval produced prepulse facilitation. Therefore, data for the 30-ms interval were analyzed separately from the other intervals (Howland et al., 2012; Ballendine et al., 2015). The data from both experiments 1 and 2 were first analyzed with repeated measures ANOVAs (drug treatment, d -GOV or l -GOV, prepulse-pulse interval and prepulse intensity as factors). Additional analysis was conducted on experiment 2 data with separate ANOVAs where d -GOV and l -GOV were analyzed separately. The data from experiment 3 was analyzed with a repeated measure ANOVA (l -GOV dose, prepulse-pulse interval and prepulse intensity as factors). Startle data were analyzed with repeated measures ANOVAs (drug treatment, d -GOV or l -GOV, and pulse block as factors for experiments 1 and 2 or l -GOV dose and pulse block as factors for experiment 3) for each experiment. Non-significant main effects and interactions are not reported.

2.4 Results

2.4.1 Disruption of PPI by apomorphine is blocked by l -GOV but not d -GOV

Startle As shown in Figure 2.1 a, rats displayed robust startle to presentation of the 120-dB tones following all treatments. A significant main effect of pulse block was observed ($F_{(1.03,19.56)}=44.15, p<0.001$) indicating habituation of startle over the testing session. None of the drug treatments affected startle (statistics for interactions not shown).

We also assessed the effects of the treatments on baseline reactivity during trials in which no stimulus was presented or the prepulses (3, 6, 12 dB) were presented alone (Figure 2.1 b). Significant main effects of both APO ($F_{(1,19)}=73.78, p<0.001$) and GOV ($F_{(2,38)}=17.07, p<0.001$)

on reactivity were found. These main effects were qualified by a significant APO by GOV interaction ($F_{(1.48,28.10)}=4.72, p=0.026$) that showed startle reactivity only significantly increased with the APO and APO + d-GOV treatments but not with the APO and l-GOV treatments.

Figure 2.1: Effects of d- and l-GOV (1.0 mg/kg) on startle and its modulation by apomorphine (APO) or MK-801 (MK).

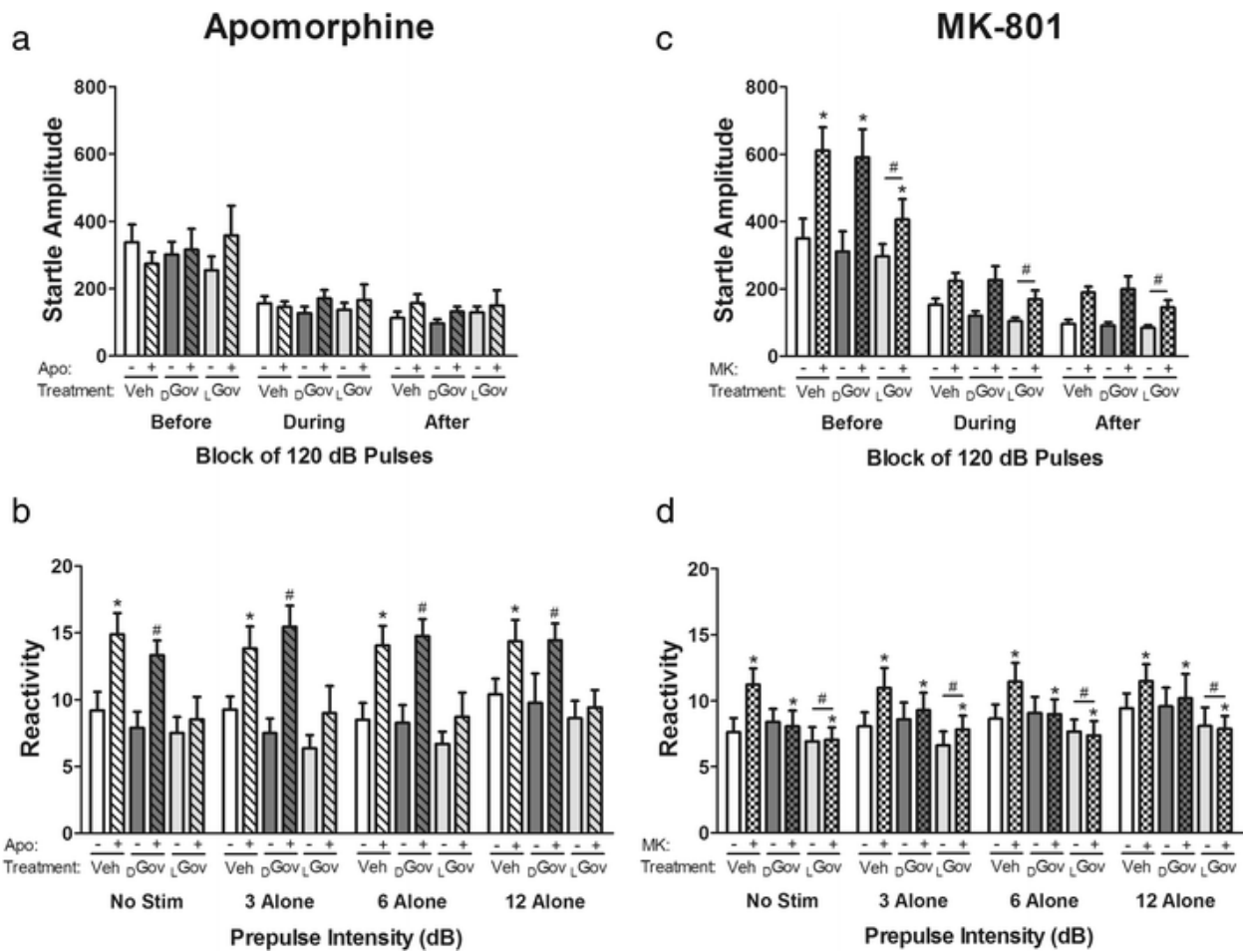


Figure 2.1: (a) Amplitude of startle (arbitrary units) when rats were treated with APO (0.2 mg/kg) and *d*- or *l*-GOV. Neither APO nor either enantiomer of GOV affected startle (arbitrary units) before, during, or after the PPI trials were presented. (b) The effects of APO and each enantiomer of GOV on reactivity during the no stimulus and 3-, 6-, and 12-dB prepulse-alone trials. Reactivity was significantly increased by Apo ($*p<0.05$). *d*-GOV had no effect on the APO-elicited increase while ($\#p<0.05$) *l*-GOV significantly reduced it. (c) Amplitude of acoustic startle (arbitrary units) when rats were treated with MK (0.15 mg/kg) and *d*- or *l*-GOV. MK-801 treatment significantly increased the startle during the first block of pulse-alone trials, but not during the second or third block ($*p<0.05$). Collapsed across MK treatment, *l*-GOV significantly decreased startle relative to *d*-GOV and vehicle treatments ($\#p<0.05$). (d) The effects of MK and each enantiomer of GOV on reactivity during the no stimulus and 3-, 6-, and 12-dB prepulse-alone trials. Regardless of GOV treatment, MK-801 treatment significantly increased reactivity ($*p<0.05$). Collapsed across MK-801 treatment, *l*-GOV significantly decreased reactivity relative to vehicle and *d*-GOV treatments ($\#P<0.05$).

PPI Rats in all treatment conditions displayed varying levels of PPI (Figure 2.2 a, b) that were determined by both prepulse-pulse interval ($F_{(2,38)}=19.45, p<0.001$) and prepulse intensity ($F_{(1.34,25.52)}=180.02, p<0.001$). The main effects of APO ($F_{(1,19)}=5.18, p=0.035$) and GOV ($F_{(2,38)}=6.10, p=0.005$) were both statistically significant. A significant APO by GOV interaction qualified these main effects ($F_{(2,38)}=5.60, p=0.007$) and revealed disrupted PPI in APO-treated animals relative to vehicle-, ι -GOV-, and APO + ι -GOV-treated animals collapsed across all prepulse-pulse intervals and prepulse intensities (Figure 2.2 a; $p<0.05$). Overall, d -GOV significantly decreased PPI relative to ι -GOV (Figure 2.2 a; $p<0.05$). Prepulse-pulse interval did not significantly interact with any of the treatment groups (all $p>0.05$) and thus the means were collapsed across prepulse-pulse interval (Figure 2.2 b). A significant APO by GOV by prepulse intensity interaction ($F_{(4,76)}=2.52, p=0.048$) revealed the effects of APO and d - or ι -GOV depended on the prepulse intensity (Figure 2.2 b). Post hoc analyses revealed that at the 3-dB prepulse intensity APO treatment significantly impaired PPI relative to vehicle, d -GOV, ι -GOV, and APO + ι -GOV treatments ($p<0.05$). Also, APO + d -GOV significantly reduced PPI relative to the vehicle treated animals ($p<0.05$). At the 6-dB prepulse intensity, APO, d -GOV, and APO + d -GOV treatments all significantly reduced PPI relative to vehicle treatment ($p<0.05$). At the 12-dB intensity, APO and APO + d -GOV treatments significantly reduced PPI relative to vehicle treatment ($p<0.05$).

When trials conducted with the 30-ms interval were examined (Figure 2.3 a), significant main effects of prepulse intensity ($F_{(1.49,28.21)}=42.26, p<0.001$) and APO were found ($F_{(1,19)}=5.12, p=0.036$). A significant APO by prepulse intensity interaction ($F_{(1.54,29.28)}=4.44, p=0.029$) followed up by post hoc analysis revealed that regardless of treatment with either GOV enantiomer, APO significantly reduced PPI relative to nonAPO-treated animals for the 3- and 6-dB intensities ($p<0.05$). Post hoc tests also demonstrated that significant PPI facilitation was observed for all treatments at the 12 dB prepulse intensity relative to the 3- and 6-dB intensities ($p<0.05$).

2.4.2 PPI impairments caused by MK-801 are reversed by ι -GOV but not d -GOV

Startle Similar to results observed for the APO-treated rats, habituation of startle to the pulse was confirmed by significant main effects of pulse block (Figure 2.1 c; $F_{(1.06,19.15)}=64.78,$

$p<0.001$). A significant main effect of MK-801 ($F_{(1,18)}=30.51, p<0.001$) and a significant MK-801 by pulse block interaction ($F_{(1.17,21.08)}=11.40, p=0.002$) revealed MK-801 treatment only significantly increased startle during the first block of startle-alone trials, but not during the second or third block ($p<0.05$). A significant main effect of GOV ($F_{(2,36)}=6.67, p=0.003$) was also found. Collapsed across MK-801 treatment, \downarrow GOV significantly decreased startle relative to \downarrow GOV and vehicle treatment ($p<0.05$).

When reactivity on trials with no stimulus or 3-, 6-, or 12- dB prepulses alone was examined (Figure 2.1d), reactivity significantly increased across all groups as prepulse-alone intensity increased ($F_{(3,54)}=4.89, p=0.004$). A significant main effect of MK-801 revealed that, regardless of GOV treatment, MK-801 treatment significantly increased reactivity ($F_{(1,18)}=4.57, p=0.046$). A significant main effect of GOV ($F_{(2,36)}=8.95, p=0.001$) was also observed. Post hoc analyses revealed that collapsed across MK-801 treatment, \downarrow GOV significantly decreased reactivity relative to vehicle and \downarrow GOV treatment ($p<0.05$).

Figure 2.2: Percent PPI displayed for the tests when rats were treated with either APO and d-GOV or l-GOV, or MK and d-GOV or l-GOV.

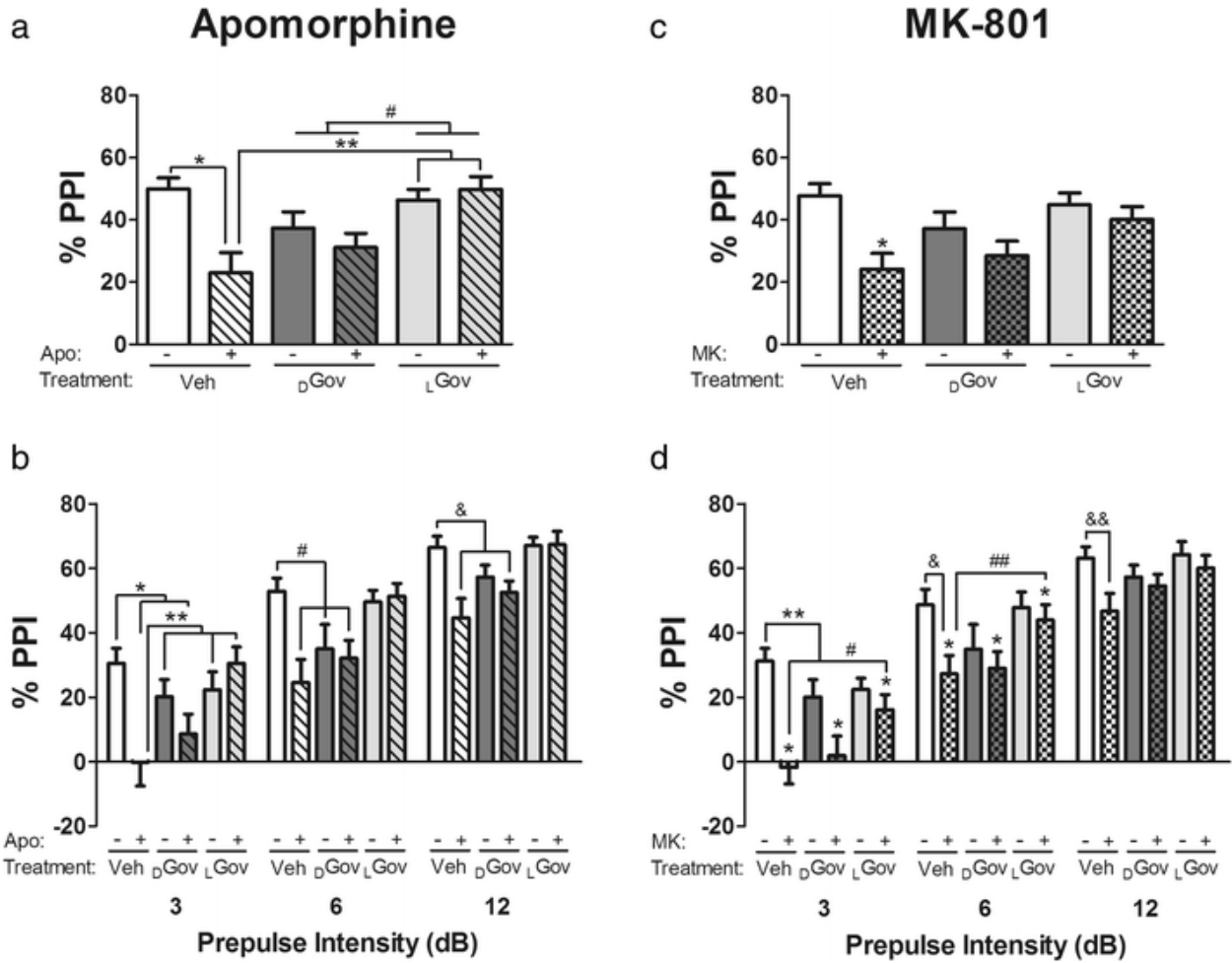


Figure 2.2: (a) Data are displayed for the average across prepulse intensity and prepulse-pulse interval with APO and d-GOV or l-GOV. APO treatment disrupted PPI relative to vehicle ($*p<0.05$), l-GOV and APO + l-GOV ($**p<0.05$) treated animals collapsed across all prepulse-pulse intervals and prepulse intensities. Overall, d-GOV significantly decreased PPI relative to l-GOV ($#p<0.05$). (b) The effects of APO and d- or l-GOV on PPI at the 3-, 6-, and 12-dB prepulse intensities averaged across prepulse-pulse interval. At the 3-dB prepulse intensity, APO treatment significantly impaired PPI relative to vehicle ($*p<0.05$), d-GOV, l-GOV, and APO + l-GOV treatments ($**p<0.05$). Also, APO + d-GOV significantly reduced PPI relative to vehicle treatment ($*p<0.05$). At the 6-dB prepulse intensity, APO, d-GOV, and APO + d-GOV treatments all significantly reduced PPI relative to vehicle treatment ($#p<0.05$). At the 12-dB intensity, APO and APO + d-GOV treatments significantly reduced PPI relative to vehicle treatment ($&p<0.05$). (c) PPI averaged across prepulse intensity and prepulse-pulse interval with MK and d-GOV or l-GOV. MK significantly reduced PPI relative to vehicle treatment ($*p<0.05$). (d) The effects of MK and d- or l-GOV on PPI at the 3-, 6-, and 12-dB prepulse intensities averaged across prepulse-pulse interval. MK treatment impaired PPI at the 3- and 6-dB prepulse intensity, but not at the 12-dB intensity ($*p<0.05$). At the 3-dB prepulse intensity, MK and MK + l-GOV treatments resulted in significantly reduced PPI relative to vehicle treatment ($**p<0.05$). MK + l-GOV treatment resulted in increased PPI relative to MK treatment alone ($#p<0.05$). At the 6-dB intensity, MK significantly reduced PPI relative to both vehicle ($&p<0.05$) and MK + l-GOV treatment ($##p<0.05$). At the 12-dB intensity, MK-801 significantly reduced PPI compared to vehicle treatment ($&&p<0.05$).

PPI Significant main effects of prepulse-pulse interval ($F_{(1.48,26.67)}=6.29, p=0.010$) and prepulse intensity ($F_{(1.49,26.74)}=216.19, p<0.001$) indicate that all animals displayed different levels of PPI for the varying levels of interval and intensity (Figure 2.2 c, d). As prepulse-pulse interval did not significantly interact with any of the treatment groups (all $p>0.05$), the remaining means and analyses were collapsed across prepulse-pulse interval. Significant main effects of MK-801 ($F_{(1,18)}=14.54, p=0.001$) and MK-801 by intensity interaction ($F_{(2,36)}=9.18, p=0.001$) were found (Figure 2.2 d). As is well established, MK-801 disrupted PPI in a manner that was significantly related to the prepulse intensity (Reijmers and Peeters 1994; Vorhees et al. 1996). MK-801 impaired PPI at the 3- and 6-dB prepulse intensity, but not at the 12- dB intensity ($p<0.05$).

In the omnibus ANOVA, the main effect of GOV ($F_{(2,36)}=3.05, p=0.060$) and MK-801 by GOV interaction ($F_{(2,36)}=2.68, p=0.082$) failed to reach significance; however, inspection of the graphical results appeared to indicate an effect of ι -GOV on MK-801 disrupted PPI. Due to increased risk of type II error as a result of multiple comparisons in the omnibus ANOVA, we performed a secondary repeated measure ANOVA which assessed the effects of each GOV enantiomer on PPI separately. Dissociable effects of the enantiomers on the MK-801- induced disruption of PPI were apparent. Inspection of the results for the ι -GOV enantiomer produced the same effects observed for the omnibus ANOVA, specifically, a main effect of MK-801 ($F_{(1,18)}=12.88, p=0.002$), a main effect of prepulse-pulse interval ($F_{(2,36)}=4.49, p=0.018$), a main effect of prepulse intensity ($F_{(2,36)}=200.12, p<0.001$), and an MK-801 by prepulse intensity interaction ($F_{(2,36)}=10.00, p<0.001$). Inspection of the ι -GOV analysis also revealed a main effect of MK-801 ($F_{(1,18)}=16.09, p=0.001$), a main effect of prepulse-pulse interval ($F_{(2,36)}=5.84, p=0.006$), a main effect of prepulse intensity ($F_{(2,36)}=169.36, p<0.001$), and a MK-801 by prepulse intensity interaction ($F_{(2,36)}=11.00, p<0.001$). Additionally, this analysis revealed a significant MK-801 by ι -GOV interaction ($F_{(1,18)}=5.74, p=0.028$) and a significant MK-801 by ι -GOV by intensity interaction (Figure 2.2 d; $F_{(1.43,25.8)}=5.29, p=0.020$). Post hoc analyses indicate that at the 3-dB prepulse intensity, MK-801 and MK-801 + ι -GOV treatments resulted in significantly reduced PPI relative to vehicle treatment ($p<0.05$). Further post hocs revealed that MK-801 + ι -GOV treatment resulted in increased PPI relative to MK-801 treatment alone ($p<0.05$). At the 6-dB intensity, MK-801 significantly reduced PPI relative to both vehicle and

MK-801 + μ -GOV treatment ($p<0.05$). At the 12-dB intensity, post hoc analysis revealed that MK-801 significantly reduced PPI compared to vehicle treatment ($p<0.05$).

Analysis of the 30-ms interval (Figure 2.3 b) revealed a significant main effect of prepulse intensity ($F_{(2,36)}=51.41, p<0.001$) and MK-801 ($F_{(1,18)}=12.25, p=0.003$). Post hoc analysis of a significant prepulse intensity by MK-801 interaction ($F_{(2,36)}=4.06, p=0.026$) indicated that, collapsed across GOV treatment, PPI facilitation was observed in the vehicle animals at the 12-dB intensity relative to the 3-dB intensity. Alternatively, MK-801 treatment collapsed across all GOV treatments resulted in a shift towards PPI facilitation at the 12-dB intensity relative to the 6-dB intensity. Further analysis of the 30-ms data revealed a significant prepulse intensity by GOV interaction ($F_{(4,72)}=2.90, p=0.028$); however, no meaningful changes in PPI produced by GOV treatment were observed at varying levels of prepulse intensity.

Figure 2.3: Percent PPI on trials with a short (30-ms) prepulse-pulse interval when rats were treated with either APO and d-GOV or l-GOV or MK and d-GOV or l-GOV.

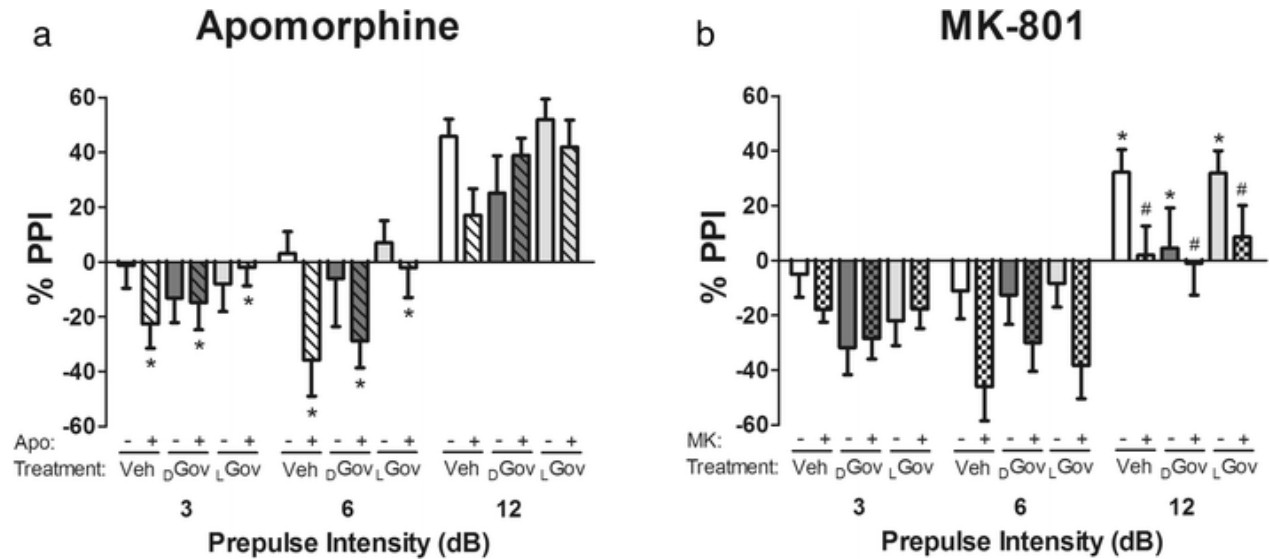


Figure 2.3: (a) APO significantly reduced PPI relative to non-APO treated animals for the 3- and 6-dB intensities (* $p < 0.05$). Collapsed across GOV treatments, PPI facilitation was observed in the vehicle animals at the 12-dB intensity relative to the 3- dB intensity (* $p < 0.05$). **(b)** MK treatment collapsed across all GOV treatments resulted in PPI facilitation at the 12-dB intensity relative to the 6-dB intensity (# $p < 0.05$).

2.4.3 Dose-dependent effects of ι -GOV on startle and PPI

Startle Consistent with results obtained for the APO and MK-801-treated rats, habituation of startle to the tone was observed (Figure 2.4 a; main effect of pulse block: $F_{(2,22)}=53.81, p<0.001$). A significant main effect of treatment ($F_{(3,33)}=5.75, p=0.003$) revealed that the 1.0 and 3.0 mg/ kg doses of ι -GOV significantly decreased startle amplitude relative to vehicle treatment ($p<0.05$).

Examination of startle reactivity to the no stimulus or 3-, 6-, or 12-dB prepulses alone (Figure 2.4 b) showed a significant main effect of treatment ($F_{(1.72,18.88)}=10.44, p=0.001$). Post hoc analyses revealed that the 1.0 and 3.0 mg/kg doses of ι -GOV produced significantly decreased reactivity to all prepulse intensities relative to vehicle treatment ($p<0.05$). Analyses further revealed that 3.0 mg/kg of ι -GOV also significantly decreased reactivity relative to the 0.3 mg/kg dose ($p<0.05$).

PPI Similar to the first two experiments, significant main effects of prepulse-pulse interval ($F_{(2,22)}=4.54, p=0.022$); prepulse intensity ($F_{(2,22)}=374.00, p<0.001$); and a significant prepulse-pulse interval by prepulse intensity interaction ($F_{(2.18,24.01)}=9.29, p=0.001$) demonstrate that all animals had varying degrees of PPI across the levels of interval and intensity (Figure 2.4 d). Prepulse-pulse interval did not significantly interact with ι -GOV dose ($p>0.05$). However, a significant main effect of treatment ($F_{(3,33)}=4.11, p=0.014$) and an interaction between treatment and prepulse intensity (Figure 2.4 d; $F_{(6,66)}=3.32, p=0.006$) were found. A significant treatment by prepulse-pulse interval by prepulse intensity interaction was also observed ($F_{(12,132)}=2.23, p=0.014$). Post hoc analyses revealed that at the 3-dB intensity, treatment with 0.3 mg/kg of ι -GOV significantly decreased PPI relative to the 1.0 and 3.0 mg/kg doses ($p<0.05$).

Analysis of the 30-ms interval trials (Figure 2.4 e) revealed a significant main effect of prepulse intensity ($F_{(1.36,14.98)}=88.66, p<0.001$) and treatment ($F_{(3,33)}=3.74, p=0.020$). Although all treatment groups showed increasing evidence of PPI as prepulse intensity increased, rats treated with 3.0 mg/kg of ι -GOV showed consistently higher PPI overall relative to animals treated with 0.3 mg/kg of ι -GOV ($p<0.05$).

Figure 2.4: Effects of l-GOV (0.3, 1.0, 3.0 mg/kg) or vehicle on startle, startle reactivity, percent PPI long-interval trials, and percent PPI short-interval trials.

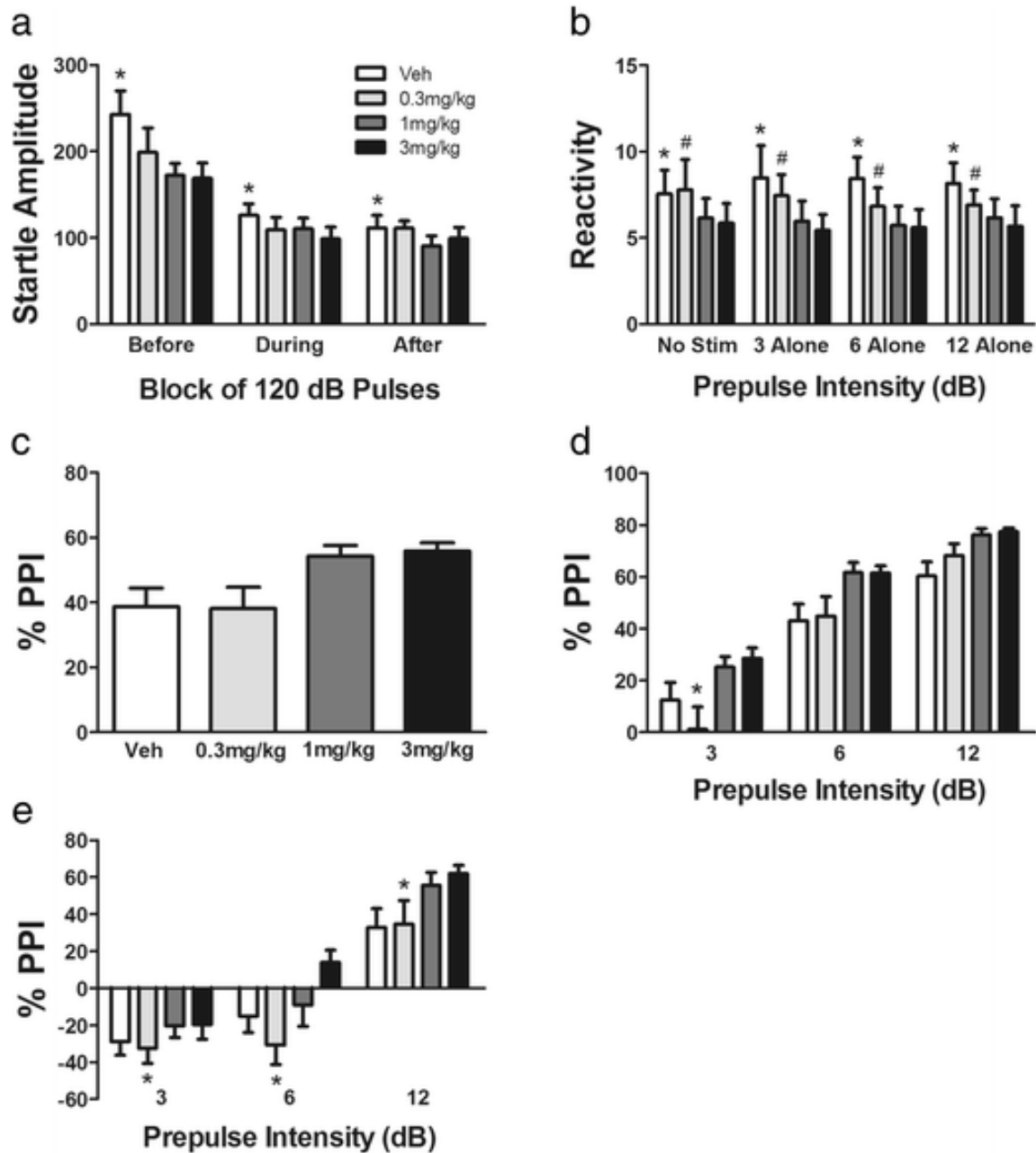


Figure 2.4: (a) Amplitude of startle (arbitrary units) when rats were treated with varying doses of l-GOV . Both the 1.0 and 3.0 mg/kg dose of l-GOV significantly decreased startle before, during, and after the PPI trials were presented ($*p<0.05$). (b) The effects of l-GOV dose on reactivity during the no stimulus and 3-, 6-, and 12-dB prepulse-alone trials. Both the 1.0 and 3.0 mg/kg dose of l-GOV significantly decreased reactivity relative to vehicle treatment during the no stimulus and all prepulse-alone trials ($*p<0.05$). The 3.0 mg/kg dose of l-GOV also significantly decreased reactivity relative to the 0.3 mg/kg dose across all trials ($\#p<0.05$). (c) Average PPI across all prepulse-pulse intervals and prepulse intensities for each dose of l-GOV . (d) The effects of each dose of l-GOV on PPI at the 3-, 6-, and 12-dB prepulse intensities averaged across prepulse-pulse interval. The 0.3 mg/kg dose of l-GOV resulted in significantly decreased PPI relative to the 1.0 and 3.0 mg/kg doses at the 3 dB intensity ($*p<0.05$). (e) Percent PPI for the 30 ms prepulse-pulse intervals when rats were treated with each dose of l-GOV . Averaged across all prepulse intensities, the 0.3 mg/kg dose of l-GOV significantly decreased PPI relative to the 3.0 mg/kg dose ($*p<0.05$).

2.5 Discussion

The present study tested the effects of the d- and l- enantiomers of GOV on PPI alone and when disrupted by APO and MK-801. APO disrupted PPI (Figure 2.2) without significant effects on startle (Figure 2.1) whereas MK-801 increased startle (Figure 2.1) and also disrupted PPI (Figure 2.2). Interestingly, both drugs increased reactivity during trials in which a pulse was not presented (Figure 2.1), effects which were blocked by l-GOV, but not d-GOV. l-GOV, but not d-GOV, blocked the disruptive effects of APO (Figure 2.2) and MK-801 (Figure 2.2) on PPI at varying prepulse intensities (3, 6, and 12 dB). As previously reported, trials with a short prepulse-pulse interval (30 ms) had low levels of PPI, particularly for trials with 3- and 6-dB prepulses (Figure 2.3). MK-801 and APO tended to reduce PPI for these trials. The enantiomers of GOV did not significantly affect PPI on short interval trials; however, d-GOV alone significantly disrupted PPI at the long-interval relative to l-GOV. Taken together, these results suggest that l-GOV functions much like atypical antipsychotic drugs in blocking the effects of APO and MK-801 on PPI (Swerdlow and Geyer, 1993).

2.5.1 The effects of apomorphine and MK-801 on PPI in Long-Evans rats

The PPI protocol employed in the present experiments used a range of intervals between prepulses and the startling pulse because previous research has shown interval-specific effects of some manipulations (Jones and Shannon, 2000; Fendt et al., 2001; Yeomans et al., 2010; Pinnock et al., 2015). Our results for the long-interval trials (50, 80, 140 ms between the prepulse and pulse) confirm the well-documented impairments of PPI caused by the direct DA D2R agonist APO and NMDA receptor antagonist MK-801 in previous studies using long prepulse-pulse intervals (Geyer et al., 2001). Previous studies have shown that hooded rats, including the Long-Evans (used here) and Lister strains are less sensitive to the disruptive effects of APO, but not MK-801, on PPI than the Sprague-Dawley and Wistar strains (Kinney et al., 1999; Swerdlow et al., 2000; Weiss et al., 2000; Qu et al., 2009). We observed a robust disruption in PPI following APO treatment. This was observed using a moderate dose of APO (0.2 mg/kg) which has been shown to impair PPI in Long-Evans rats in some studies (Howland et al., 2004) but not others (Swerdlow et al., 2001a). Short-interval trials (30-ms prepulse-pulse interval) were characterized by lower PPI than long-interval trials, particularly for the trials with 3- and 6-dB

prepulses. We and others have observed this pattern previously (Swerdlow et al., 2000; van den Buuse and Gogos, 2007; Jones et al., 2010; Howland et al., 2012; Ballendine et al., 2015).

Interestingly, startle was in fact increased during short interval prepulse-pulse trials (i.e., a form of prepulse facilitation) following MK-801 and APO consistent with previous studies using MK-801 (Al-Amin and Schwarzkopf, 1996; Brosda et al., 2011) or ketamine (Mansbach and Geyer, 1991) with short intervals between prepulse and pulse.

MK-801 (0.15 mg/kg) significantly increased the startle to the 120-dB pulse throughout the test session, an effect that has been reported previously following the same dose in Sprague Dawley and Wistar rats (Varty et al., 1999 but also see Wiley et al., 2003). Interestingly, startle reactivity on no-stimulus and prepulse-alone trials was significantly enhanced by both APO and MK-801. Others have reported no effect of APO on reactivity in Long-Evans rats (Swerdlow et al., 2001a) although increased reactivity has been noted in Sprague-Dawley rats (Swerdlow et al., 2001a, 2004) and C57BL/6 mice (Yee et al., 2004b). The effects of NMDA receptor antagonists on prepulse-elicited reactivity have not been consistent with the enhancement produced by APO (Yee et al., 2004a; Yee and Feldon, 2009) although to the best of our knowledge, the effects of MK-801 on prepulse-elicited reactivity in rats have not been reported previously. The increase in reactivity caused by APO and MK-801 may reflect a generalized increase in locomotor behaviour caused by these drugs. Interestingly, ι -GOV has been shown previously to block AMPH-induced locomotion (Lapish et al., 2014). Therefore, testing the effects of ι -GOV on locomotor behaviour induced by APO or MK-801 in an open field may show a generalized effect of ι -GOV on locomotor behaviour caused by a range of psychotomimetic drugs.

2.5.2 Selective effects of the d- and l- enantiomers of GOV on the disruptions in PPI induced by apomorphine and MK-801

Previous studies have demonstrated that ι -GOV induces deficits in conditioned avoidance responding and attenuates AMPH-induced locomotion (Lapish et al., 2014). In contrast, d -GOV has no such effects; however, it improves working memory and temporal order memory. Despite these differences in cognitive and behavioural effects, both enantiomers act to improve social interaction and latent inhibition in animal models of schizophrenia (Lapish et al., 2012, 2014).

The present results are novel and show that l-GOV blocks the disruptive effect of either APO or MK-801 on PPI. As well, l-GOV reduces the APO- and MK-801-induced increases in reactivity. d-GOV has no effect on these measures, although it did reduce PPI for long-interval trials. While the mechanism underlying the reduction in PPI following d-GOV will be difficult to discern, it may relate to the enantiomer's unique effects on DA release and receptor antagonism (Lapish et al., 2014b). Well-documented side effects of typical antipsychotics are extrapyramidal symptoms (EPS) which include Parkinsonism, dystonia, akathisia, and tardive dyskinesia (Porsolt et al., 2010). In rodents, catalepsy is a common behavioural measure used to assess the potential for a neuroleptic drug to induce EPS (Porsolt et al., 2010; Lapish et al., 2014b). In a previous study l-GOV , but not d-GOV , induced catalepsy in a dose-dependent manner; however, minimal effects were observed at 0.3 and 1.0 mg/kg with an increase in immobility at the 3.0 mg/kg dose. This effect is consistent with its profile as a putative antipsychotic and D2 antagonist (Lapish et al., 2014b). In the dose-response study (Figure 2.4), 1.0 and 3.0 mg/kg of l-GOV reduced startle reactivity, which may reflect a generalized effect on locomotor activity consistent with effects reported for haloperidol and clozapine by Hoffman et al. (1993). However, dose-dependent changes in PPI were relatively subtle, while a non-significant increase in PPI was noted following higher doses of l-GOV , an effect also found with haloperidol (Hoffman et al., 1993). Disrupted PPI following APO treatment has been linked to activation of the mesolimbic DA system (Geyer et al., 2001). Consistent with this theory are findings showing that typical antipsychotic drugs such as haloperidol, which are potent D2 receptor antagonists, block the effects of APO on PPI (Swerdlow and Geyer, 1993; Geyer et al., 2001). Thus, the effects of l-GOV on the APO-induced disruption of PPI may be attributable to its demonstrated similarity to typical antipsychotics, as an antagonist with high affinity for D2 receptor and its effects in behavioural assays such as conditioned avoidance responding (Lapish et al., 2014b). In keeping with this theory, the failure of d-GOV to block the effect of APO on PPI may be related to its relatively lower affinity for D2 receptors.

In contrast to the reversal of APO-induced PPI impairment, typical antipsychotics such as haloperidol do not block PPI impairment when induced by MK-801 (Keith et al., 1991; Hoffman et al., 1993; Bast et al., 2000). Therefore, the D2 receptor antagonism caused by l-GOV is not a likely explanation for the reversal of the MK-801-induced PPI impairment. In some studies,

atypical antipsychotics such as clozapine block the effects of acute MK-801 (or other NMDA receptor antagonists) on PPI (Bakshi et al., 1994; Geyer et al., 2001; Bubeníková et al., 2005 but see Hoffman et al., 1993; Bast et al., 2000) raising the possibility that serotonin and/or muscarinic receptor mechanisms may be involved. At psychotomimetic doses, non-competitive NMDA receptor antagonists such as MK-801 disrupt neural circuits by reducing GABAergic transmission which leads to disinhibition of pyramidal neurons and increased midbrain DA efflux (Laruelle et al., 1999; Kegeles et al., 2000; Balla et al., 2001, 2003; Homayoun and Moghaddam, 2007; Vinson and Conn, 2012). μ GOV's reversal of the MK-801-induced PPI impairment may be explained by its unique ability to simultaneously enhance DA efflux in the PFC and block D2 receptors (Lapish et al., 2014b). Stimulation of D1 receptors increases inhibitory neurotransmission in the PFC while D2 receptor stimulation decreases it (Seamans et al., 2001; Gorelova et al., 2002). Differential DA signaling is regulated in a concentration dependent manner (Trantham-Davidson et al., 2004). D1 receptor signaling is proposed to cause a prolonged increase in IPSC through activation of adenylyl cyclase (AC) and protein kinase A (PKA), which inhibit K⁺ channels in parvalbumin-containing interneurons. Blocking D2 receptors in the presence of high DA concentration increases inhibitory postsynaptic currents, an effect that is prevented by D1 receptor antagonists (Gorelova et al., 2002). Thus, D2 receptor signaling may occlude D1 receptor signaling during periods of high DA concentration and this occlusion can be prevented with D2 antagonism (Gorelova et al., 2002). As μ GOV blocks D2 receptors while increasing DA efflux, the resulting increase in D1 receptor binding and signaling may lead to increased PKA and ultimately, increased excitability in parvalbumin-containing interneurons to counteract the effects of MK-801. Such a mechanism is supported by findings that NMDA receptor antagonist-induced PPI deficits are reversed by the GABA receptor agonist baclofen (Bortolato et al., 2004) and D1 receptor agonist A77636 (Bubenikova-Valesova et al., 2009). Additionally, infusion of the GABA-A channel blocker picrotoxin into the mPFC via intracranial cannulae disrupts PPI and is reversed by pretreatment with haloperidol in Wistar rats (Japha and Koch, 1999). It is important to note that this effect may be strain dependent as it was not replicated in Lister hooded rats (Pezze et al., 2014). Activation of D1 receptors also stimulates the translocation of NMDA receptors to the postsynaptic membrane (Dunah et al., 2004) and secondary messengers which phosphorylate NMDA receptors subunits to potentiate

the NMDA-evoked response (Missale et al., 2006) providing additional mechanisms through which l-GOV may reverse the effects of NMDA receptor antagonism. d-GOV , which is not a potent D2 receptor antagonist (Lapish et al., 2014b), lacks the ability to restore the PPI deficits in both drug conditions.

2.6 Conclusion

These data are consistent with the potential use of enantiomers of GOV as treatments for schizophrenia. l-GOV , a D2 receptor antagonist which increases DA efflux, was effective in restoring deficits in PPI induced by MK-801 or APO to control levels. In contrast, d-GOV , the enantiomer associated with cognitive enhancement, did not reverse the disruption of PPI by either drug. A dose-response study revealed l-GOV reduced startle amplitude, reactivity, and PPI in the dose-dependent manner.

CHAPTER 3

PROSPECTIVE ANALYSIS OF THE EFFECTS OF MATERNAL IMMUNE ACTIVATION ON RAT CYTOKINES DURING PREGNANCY AND BEHAVIOUR OF THE MALE OFFSPRING RELEVANT TO SCHIZOPHRENIA²

3.1 Abstract

Influenza during pregnancy is associated with the development of psychopathology in the offspring. We sought to determine if maternal cytokines produced following administration of viral mimetic polyI:C to pregnant rats were predictive of behavioural abnormalities in the adult offspring. Timed-pregnant Sprague-Dawley rats received a single intravenous injection of 4 mg/kg polyI:C or saline on GD15. Blood was collected for serum analysis of cytokine levels with ELISA. Male offspring were tested in a battery of behavioural tests at adulthood and behaviour correlated with maternal cytokine levels. Maternal serum levels of CXCL1 and IL-6, but not TNF- α or CXCL2, were elevated in polyI:C-treated dams. PolyI:C-treated dams experienced post-treatment weight loss and polyI:C pups were smaller than controls at postnatal day 1. Various behaviour alterations were seen in the polyI:C-treated offspring. Male polyI:C offspring had enhanced MK-801-induced locomotion, and reduced sociability. PolyI:C offspring failed to display crossmodal and visual memory, and oddity preference was also impaired. Set-shifting, assessed with a lever-based operant conditioning task, was facilitated while touchscreen-based reversal learning was impaired. Correlations were found between maternal

² **Lins BR**, Hurtubise JL, Roebuck AJ, Marks WN, Zabder NK, Scott GA, Greba Q, Dawicki W, Zhang X, Rudulier CD, Gordon JR, Howland JG (2018) Prospective analysis of the effects of maternal immune activation on rat cytokines during pregnancy and behaviour of the male offspring relevant to schizophrenia. *eNeuro*.0249-18.2018.

Lins organized and led the experiments, collected and analyzed data, and co-wrote the manuscript; Hurtubise, Roebuck, Marks, Zabder, Scott, and Greba collected behaviour data; Zhang and Rudulier performed ELISAs; Dawicki and Gordon advised on immunology topics; Howland supervised the experiments and co-wrote the manuscript. This manuscript is peer-reviewed and published.

serum concentrations of CXCL1, acute maternal temperature and body weight changes, neonatal pup mass, and odd object discrimination and social behaviour. Overall, while the offspring of polyI:C-treated rats displayed behaviour abnormalities, maternal serum cytokines were not related to the long-term behaviour changes in the offspring. Maternal sickness effects and neonatal pup size may be better indicators of later effects in the offspring.

3.1.1 Significance statement

Psychiatric pathology is complex, poorly understood, and often results in chronic illness. Many psychiatric conditions are believed to occur as a result of genetic and environmental factors. Gestational adversity such as inflammation in pregnancy may act as a priming experience for the later emergence of psychopathologies, and accurate identification of risk factors may advise early interventions. We sought to characterize long-term behaviour effects in the offspring of rats exposed to an inflammatory event during pregnancy and relate these effects to the serum levels of relevant cytokines CXCL1, IL-6, and TNF- α . Our results suggest that these maternal cytokines are not strongly related to offspring behaviour outcomes, and other measures may have greater value as predictors of behaviour outcomes.

3.2 Introduction

Inflammation during pregnancy is associated with increased risk of various psychopathologies in the offspring (Brown et al., 2004a; Brown, 2006; Fineberg and Ellman, 2013). The association between inflammation and psychiatric illness was initially demonstrated through epidemiological studies where influenza outbreaks preceded an increase in SSDs as cohorts that were *in utero* during the epidemic reached adulthood (Brown, 2012). Inflammation in pregnancy has since been linked to additional pathologies in the offspring including bipolar disorder and autism (Atladóttir et al., 2010, 2012; Parboosing et al., 2013; Jiang et al., 2016; Scola and Duong, 2017). Heterogeneity of the pathogens (viral, bacterial, parasitic) associated with psychiatric outcomes suggests the maternal immune response may mediate the effects on the developing offspring (Brown and Patterson, 2011). This hypothesis has been corroborated through prospective studies where maternal serum levels of IL-8/CXCL8, a cytokine with cellular attracting properties in the chemokine family, were elevated during the second trimester

in pregnancies where the offspring developed an SSD (Brown et al., 2004b). Subsequent research implicated exposure to IL-8 *in utero* with abnormalities in the offspring's brains including increased ventricular cerebrospinal fluid and decreased cortical volumes (Ellman et al., 2010). Increased TNF- α in late pregnancy has also been linked to schizophrenia in the offspring (Buka et al., 2001). A study in humans demonstrated retrospective estimations of maternal IL-6 levels during pregnancy using newborn functional brain connectivity determined by magnetic resonance imaging (MRI) and gestational IL-6 levels were predictive of performance in a working memory task at 2 years old (Rudolph et al., 2018). A second study that followed human pregnancies and offspring at 6 months of age found maternal inflammatory cytokines (IL-6, TNF- α , MCP-1) mediated an effect of maternal depressive symptoms on negative affect in the offspring (Gustafsson et al., 2018).

Systemic treatment of pregnant rodents or nonhuman primates with an immune stimulant such as the synthetic double-stranded RNA molecule polyI:C induces various brain changes in the offspring reminiscent of psychiatric illness (Meyer et al., 2009; Piontkewitz et al., 2012; Meyer, 2014). PolyI:C increases pro-inflammatory cytokines such as IL-1 β , IL-6, CXCL1 (rodent homologue of IL-8), and TNF- α in maternal circulation (Meyer et al., 2006; Hsiao and Patterson, 2011; Ballendine et al., 2015). A mouse study showed a causal role for IL-6 in the development of the offspring's psychopathology as administration of IL-6 alone resulted in abnormal offspring behaviour and abnormalities could be prevented with concomitant administration of an IL-6 antibody (Smith et al., 2007). However, few studies have prospectively analyzed the relationship between increased maternal cytokines during pregnancy and behaviour of the offspring. In one study with rats, dams that lost weight following polyI:C had significantly higher serum TNF- α than those that gained weight. Offspring of dams that lost weight had reduced sucrose preference but no significant changes in PPI or locomotor responses to AMPH or MK-801 (Missault et al., 2014).

Improved understanding of the relationship between maternal serum cytokines and offspring phenotype has potential to impact psychiatric disease prevalence through screening and early intervention, yet there is a lack of prospective data correlating maternal inflammation with offspring behavioural phenotypes (Jiang et al., 2016). The present study assessed the effects of maternal polyI:C on acute cytokine elevations in the dams and subsequent behavioural

abnormalities in the offspring. The cytokines analysed were based on those previously indicated as relevant in the literature: IL-6, IL-8/CXCL1, and TNF- α as well as CXCL2 as a negative control (Brown et al., 2004b; Smith et al., 2007; Ellman et al., 2010; Missault et al., 2014; Ballendine et al., 2015; Scola and Duong, 2017). To assay behaviour related to the positive symptoms of schizophrenia we used locomotor activity in response to a novel environment and the NMDA receptor antagonist MK-801 (Zuckerman and Weiner, 2005; Howland et al., 2012; Giovanoli et al., 2013). For negative symptoms we used a spontaneous test sociability test (Bitanirwe et al., 2010). Cognitive impairment was assessed using PPI of the acoustic startle response (Meyer et al., 2009; Howland et al., 2012; Ballendine et al., 2015), a crossmodal recognition memory battery (CMOR) (Winters and Reid, 2010; Ballendine et al., 2015), a spontaneous oddity task (Bartko et al., 2007a), and two operant conditioning procedures which assess visual discrimination, strategy set-shifting, and reversal learning (Zhang et al., 2012; Ballendine et al., 2015; Bryce and Howland, 2015). Our hypothesis was that maternal pro-inflammatory cytokines would correlate with behavioural deficits in the offspring with higher cytokine concentrations relating to a more severe behaviour phenotype.

3.3 Materials and methods

3.3.1 Animals

Timed-pregnant Sprague-Dawley rat dams (n=43) arrived at the animal holding facility on GD7. Dams were singly housed in standard polypropylene cages in a temperature controlled (21°C) colony room on a 12/12 h light/dark cycle (lights on at 07:00 h) with food (Purina Rat Chow) and water available *ad libitum*. Following arrival, dams were left undisturbed until treatment on GD15. All procedures were carried out during the light phase and behavioural experiments were conducted on adult male offspring (n=121). Experimental procedures were approved by the University of Saskatchewan Animal Research Ethics Board.

3.3.2 Maternal treatments and blood samples

Maternal treatment generally followed previously established protocols in Long Evans rats (Figure 3.1 A). On GD15, dam weight and rectal temperature were recorded. Dams were anesthetized with isoflurane (5% induction, 2.5% maintenance) for <10 minutes and received a

single intravenous (i.v.) tail vein injection of either 0.9% saline or polyI:C (4 mg/kg, high molecular weight, InVivoGen, San Diego, CA, USA). Dams were anesthetized a second time as described above 3 h following initial treatment and a blood sample (<1.5 mL total and <6% total blood volume) was drawn using a sterile catheter (BD Insyte™ Autoguard™, 24 GA 0.75 IN 0.7 x 19 mm, REF 381412) from the opposite tail vein used to inject polyI:C. Warm physiological saline was administered once following polyI:C or saline treatment (3 mL), and a second time after blood collection (volume = the blood sample). Blood samples coagulated at room temperature for 1 h and spun at 10,000xG for 5 min to separate the serum. Serum was pipetted and stored at -80 °C until analysis and ELISAs were performed for CXCL1 (GRO α /KC; R&D Systems, Minneapolis, MN), CXCL2 (GRO β /MIP-2; R&D Systems), IL-6 (PeproTech, Rocky Hill, NJ), and TNF- α (PeproTech) per the manufacturer's instructions. Difficulty with blood collection of 3 polyI:C treated dams resulted in small serum volumes collected for these rats and they were not included in IL-6 ELISA, and 1 was also excluded from TNF- α ELISA. While the offspring from these dams were included in behavioural testing, their behaviour could not be correlated with these cytokine levels.

Aside from maternal weight and temperature monitoring 8, 24, and 48 h following treatment, dams were undisturbed for the remainder of their pregnancy. Of the original n=43 dams, 7 were euthanized within 48 h following polyI:C injection due to hypothermia. Four additional dams experienced body temperature below 36°C but otherwise showed alert behaviour and were given access to a warming pad on their home cage for 24 h until their temperature returned to normal. Three dams did not produce viable litters. Ultimately, offspring from a total of n=33 litters were included (n=17 polyI:C treated dams and n=16 saline treated dams). On postnatal day (PND) 1, litters were weighed, sexed, and culled to a maximum of 10 (6 males where possible). On PND23, pups were weaned and housed in same-sex sibling groups of 2 or 3 in standard housing as previously described.

A

GD0 Start of Pregnancy GD15 Maternal Treatment GD21/PND0 Birth of Pups PND56-112 Offspring Behaviour Tests

B

PPI → CMOR → Sociability → OSST
Oddity Discrimination → MK-801 induced Locomotor Activity → PD/RL

C Maternal Body Weight

Time (h)	Saline (% Change)	PolyI:C (% Change)
0 h	100	100
8 h	~96*	~95*
24 h	~100*	~95
48 h	~104*	~98

D Maternal Body Temperature

Time (h)	Saline (°C)	PolyI:C (°C)
0 h	~37.7	~37.7
8 h	~37.9	~37.5
24 h	~37.4	~37.4
48 h	~37.3	~37.2

E Maternal Serum Cytokine Concentrations

Cytokine	Saline (pg/mL)	PolyI:C (pg/mL)
CXCL1	~200	~5500*
CXCL2	~100	~100
IL-6	~200	~500*
TNF-a	~100	~200

F Pup Mass PND1

Group	Weight (g)
Saline	~8.5
PolyI:C	~7.5*

Figure 3.1: [A] Schematic illustrating the timeline of maternal treatment and offspring behaviour testing. [B] Schematic illustrating the division and order of male offspring behaviour testing. [C] Maternal weight loss was observed following the administration of polyI:C or saline. At all three time points measured (8, 24, 48 h), dams treated with polyI:C had a significantly lower percent of their original body weight than those that received saline. [D] Maternal body temperature was slightly elevated in the polyI:C treated dams 8 hours following injection, yet this effect was not significant. [E] Maternal serum cytokine concentrations were determined using ELISA and revealed a significant increase in CXCL1 and IL-6 3 h after polyI:C treatment. No differences were seen for CXCL2 or TNF- α . [F] On postnatal day 1, the pups (males and females combined) of dams treated with polyI:C weighed significantly less than those of the dams that received saline.

3.3.3 Behavioural Testing

Behavioural tests were conducted according to published protocols or modified from published protocols. Typically, 2 males per litter were used in each behaviour task. An exception is PPI where 3 male offspring per litter were included, except for the two largest litters where 4 and 5 were included. To account for the non-independent relationship between littermates, effects were averaged across siblings to produce one value per litter.

Rats were handled for a minimum of 3 sessions prior to behaviour testing. Handling included exposure to investigators and emphasized picking up and moving the rats until these motions could be carried out with ease, as well as habituation to travel by cart between the animal housing and behaviour testing locations. All work with the rats including husbandry and behaviour testing occurred during the light phase (07:00-19:00 hrs) with the majority of behaviour testing performed between 08:00-17:00. Testing began at 8 weeks of age (young adulthood) and was completed by 15 weeks of age. All offspring were first tested for PPI before being further divided into two groups for subsequent tests (Figure 3.1 B). One group (n=68) completed CMOR and sociability before being assigned to complete either the operant set shifting task (OSST, n=37) or touchscreen pairwise discrimination and reversal learning (PD/RL, n=38). The second group of randomly selected male rats (n=30) from the same litters were tested in oddity discrimination followed by MK-801 induced locomotor activity. Ethanol (40%) was used to clean all behaviour testing equipment between rats.

PPI: PPI measures the percent attenuation of motor response to a startling tone when that tone is preceded by a brief prepulse (Figure 3.2 A). Two SR-LAB startle boxes (San Diego Instruments, San Diego, CA, USA) were used. Each session had a constant background noise (70 dB) and began with 5 min of acclimatization, followed by 6 pulse-alone trials (120 dB, 40 ms). Pulse-alone (6), prepulse + pulse (36) and no stimulus (6) trials were then presented in a pseudorandom order, followed by 6 additional pulse-alone trials. Prepulse + pulse trials began with a 20 ms prepulse of 3, 6, or 12 dB above background (70 dB). Prepulse–pulse intervals (time between the onset of the prepulse and the 120-dB pulse) were short (30 ms) or long (80 ms). The inter-trial interval varied randomly from 3 to 14 s (Meyer et al., 2009; Howland et al., 2012; Ballendine et al., 2015).

Sociability Task: The testing apparatus was a rectangular arena (150 x 40 cm) of black corrugated plastic divided into three compartments, one middle start compartment (30 x 40 cm) and two ‘stranger’ compartments on either side (60 x 40 cm, see Figure 3.3 A). The walls dividing the middle compartment from the stranger compartments were clear Plexiglas (extended 12 cm from each wall leaving a 16 cm opening to move between compartments) and removable black opaque barriers which, when inserted, prevented entry into the stranger compartments. Each stranger compartment contained a circular mesh cage (18 cm diameter, 20 cm height) with hinged lid (3/4” plywood, painted matte black). The height of the cage was extended 20 cm with vertical metal rods to discourage climbing. The task began with 10 min habituation with the barriers removed. The test rat was then contained in the middle section with the barriers in place and a stranger rat was placed in one of the mesh cages. The barriers were removed, and the test rat explored for an additional 10 min. Interaction was scored when the face of the rat was oriented toward the holding cage at a maximum distance of 2 cm. Data were manually scored with a stopwatch by a blinded investigator and locomotor activity was recorded with EthoVision software. All stranger rats were sex, age, and treatment matched to the test rat (Bitanirwe et al., 2010).

Locomotor Activity: The apparatus was a square arena (40 x 40 x 60 cm) made of black corrugated plastic (Figure 3.3 D). A camera mounted to the ceiling recorded all activity and EthoVision software was used to track activity. Rats were tested 4 at a time, with each rat placed in 1 of 4 separate arenas for 30 min of habituation. Immediately following, rats were administered MK-801 (0.2 mg/kg; i.p.; Howland et al. 2012) and placed back into the arena for an additional 120 min. Activity was recorded with Noldus Ethovision XT 11.5 software (Zuckerman and Weiner, 2005; Howland et al., 2012; Giovanoli et al., 2013).

Visual, Tactile, and CMOR: This task uses spontaneous exploratory behaviour to assess visual memory, tactile memory, and visual-tactile sensory integration (Winters and Reid, 2010; Jacklin et al., 2012). The testing apparatus was a Y-shaped maze with 1 start arm and 2 object arms (10 × 27 cm) made of white corrugated plastic (Figure 3.4 A). A white plastic guillotine-style door separated the start arm from the object arms, and Velcro at the distal end of the object arms fixed objects in place. A removable, clear Plexiglas barrier could be inserted in front of the objects. A tripod positioned above the apparatus held a video camera that recorded the task activity. Rats

were habituated to the apparatus twice for 10 min. Lighting alternated during habituation between white light (used during visual phases) and red light (used during tactile phases) for 5 min each with the order counterbalanced, and the clear barriers were in place for one day of habituation and removed for the other with order counterbalanced between all rats. Test days consisted of a 3 min sample phase with two identical copies of an object attached with Velcro to the maze, a 60 min delay, and then a 2 min test phase with a third copy of the original object and a novel object placed in the maze. Rats began each phase in the start arm; the guillotine door was opened and closed once the rat entered the object arms. This task consisted of 3 distinct tests performed on 3 separate days; consistently in the following sequence: tactile memory (day 1), visual memory (day 2) and crossmodal memory (day 3). Red light illuminated the tactile phases allowing the rats' behaviour to be recorded while preventing the rats' visual assessment of the objects and the removal of the clear barriers allowed for tactile exploration. White light was used during visual phases, but clear Plexiglas barriers in front of the objects prevented tactile exploration. CMOR had a tactile sample phase (red light, no barriers) and a visual test phase (white light, clear barriers). Recognition memory was defined as significantly greater exploration of the novel object than the familiar object. Behaviour recordings were manually scored with a stopwatch by investigators blind to the treatment status of the rats and identity of the objects (Winters and Reid, 2010; Ballendine et al., 2015).

Oddity Discrimination: The testing apparatus was a square arena (60 x 60 x 60 cm) constructed of white corrugated plastic with Velcro in each of the 4 corners. Two days of habituation to the arena (10 min sessions) preceded the test day. On test day, 3 identical objects made of glass or plastic and one different, or 'odd' object were fixed to the Velcro locations (Figure 3.4 C) and the rats' activity were recorded for 5 min using a video camera mounted to the ceiling. Object exploration times were manually scored using a stopwatch by an investigator blind to the treatment status of the rats (Bartko et al., 2007a). Object examination was counted when a rat's face was oriented toward the object at a maximum distance of 2 cm.

OSST: Eight operant conditioning chambers (MedAssociates Systems, St. Albans, VT, USA) in sound-attenuating cubicles were used. The chambers contained two retractable levers and two stimulus lights positioned on either side of a food port (Figure 3.5 A) used to deliver food rewards (Dustless Precision Pellets, 45 mg, Rodent Purified Diet; BioServ, Frenchtown, NJ). A

100 mA house light illuminated the chamber. Sessions began with levers retracted and the chamber in darkness (inter-trial state), with the exception of lever training days in which the trial began with levers exposed to allow for baiting with ground reward pellets. Rats were tested once each day. *Lever training.* Rats were trained to press the levers as described previously and immediately after reaching criterion, side preference was determined. *Visual-cue discrimination.* Rats were trained to press the lever indicated by a stimulus light illuminated above it. Trials began with an illumination of one stimulus light, followed 3 s later by the house light and insertion of both levers. A correct press of the lever underneath the illuminated stimulus light caused retraction of both levers and the delivery of a reward pellet. An incorrect press returned the chamber to the inter-trial state with no reward. *Strategy set-shift.* The visual-cue rule from the previous stage was reinforced with 20 trials where the rat was required to press the lever below the illuminated stimulus light. Subsequently, rats were required to change their response from the visual cue to a spatial cue (the lever opposite to their side preference, regardless of whether the stimulus light was illuminated) to receive a reward pellet. *Reversal learning.* Rats were required to press the lever opposite to the one rewarded during set-shifting. Criterion was 10 consecutive correct responses for each testing day (Floresco et al., 2008; Zhang et al., 2012; Thai et al., 2013; Ballendine et al., 2015).

PD/RL: Eight touchscreen-equipped operant conditioning chambers (Bussey-Saksida Touch Systems, Lafayette Instrument Company, Lafayette, IN, USA) in sound attenuating cubicles were used. The chambers were trapezoidal in shape with the wider end consisting of a touchscreen monitor ($30.5 \times 24.1 \times 8.25$ cm, see Figure 3.5 D). Opposite the monitor was a food port for the delivery of food rewards. A roll-out shelf above the chamber contained a reward magazine and an overhead camera which provided a live feed of activity via output to an external monitor. The touchscreen monitor was covered with a polycarbonate mask with 2 rectangular windows that prevented contact with locations on the touchscreen irrelevant to the task. A response shelf extended 7 cm from the screen, was located below the touchscreen windows, and prevented unintentional touchscreen access. All training and testing was conducted per the manufacturer's instructions and utilized the ABET II software that accompanied the chambers. *Pretraining:* All protocols closely followed those recommended by the manufacturer and are previously published (Bryce and Howland, 2015). Rats were habituated to the chamber on 2

consecutive days with 5 reward pellets in the food port and the house light illuminated for 30 min. Criterion was reached if all pellets were consumed within 30 min. On the first day of task training, one food pellet was delivered every 30 s, as signified by a tone and illumination of the food port. During this stage of training, one of the touchscreen windows was illuminated pseudorandomly such that the same window was not illuminated for more than two consecutive trials. If the rat touched the illuminated screen, 3 reward pellets were delivered. In all stages of training and testing, each trial was preceded by a 20 s inter-trial interval that was initiated once the rat's nose entered the illuminated food port. During the second stage of training, the rat was required to nose poke the illuminated touchscreen window to receive a reward. Following this stage, the rat was required to nose poke into the illuminated port to initiate the illumination of a touchscreen window. Again, a reward was delivered if the rat then nose-poked the illuminated window. Criterion for these three stages was 100 trials in 1 h. Like the previous stages of training, the final stage of pretraining required initiation of the trial and the touching of the illuminated window to receive a food reward. However, touching the unilluminated window resulted in a 5 s timeout followed by the inter-trial interval. Trials that were incorrectly completed were followed by correction trials, whereby the same window was repeatedly illuminated until the correct selection was made and a food reward was delivered. Criterion was 100 trials completed in 1 h with a minimum of 80% correct for 2 consecutive days. Once criterion was reached, training on the full version of the task began. *Pairwise Discrimination:* Pairwise discrimination involved presentation of two distinct black and white images, one in each window of the screen. Each image could be presented in either location. One image was always correct, with its selection resulting in a food reward, regardless of its location (S+) while the other was always incorrect (S-). A correct choice resulted in presentation of a tone and illumination of the food port. An incorrect choice resulted in a 5 s delay followed by a correction trial. Rats repeated visual discrimination training daily until they reached a criterion of 100 trials completed in 1 h with $\geq 85\%$ correct for 2 consecutive days. *Reversal Learning:* Reversal learning occurred following successful completion of pairwise discrimination. The protocol for reversal learning was identical to pairwise discrimination except the previously unrewarded stimulus (S-) was now the correct choice (S+), and the previous correct choice was now

unrewarded and punished with a 5 s time out. Criterion was reached when the rat completed 100 trials in 1 h with $\geq 85\%$ correct for 2 consecutive days.

3.3.4 Statistical Analyses

A between-subjects design was used, and analyses were conducted with independent samples t-tests and ANOVAs using Statistical Package for the Social Science version 22 (IBM, Armonk, NY). Significant Time by Treatment interactions in Locomotor activity and maternal body weight were further analyzed with *a priori* pairwise comparisons to compare saline and polyI:C groups at each ordinal timepoint. Outliers were defined as having a performance metric falling more than 2 standard deviations from the mean and these were removed from analysis on a case by case basis.

Sphericity violations were accounted for using the Greenhouse-Geisser adjustment. The use of one- and two-tailed tests is specified for each task. As mentioned, there is evidence that siblings are less variable than unrelated rats (Zorrilla, 1997; Lazic, 2013; Giovanoli and Meyer, 2013b) so sibling effects were averaged to produce one value per litter. Bivariate correlations were used to analyze the relationships between maternal serum concentrations of inflammatory cytokines, other acute sickness measurements, and offspring behavioural task performance and the False Discovery Rate correction (Benjamini-Hochburg) was applied to grouped families of multiple comparisons. *P* values ≤ 0.05 were considered significant.

3.4 Results

3.4.1 Effects of polyI:C treatment on the pregnant dams and neonatal offspring

PolyI:C treatment significantly affected acute maternal body weight changes measured as % change from baseline immediately prior to treatment (Figure 3.1 C). A repeated measures Analysis of Variance (ANOVA) revealed significant effects of Time ($F_{(1.51,46.90)}=56.17$, $p<0.001$), Treatment ($F_{(1,31)}=30.52$, $p<0.001$) as well as a Time by Treatment interaction ($F_{(1.51,46.90)}=16.91$, $p<0.001$) in a repeated measures ANOVA. The polyI:C-treated dams had reduced body weight compared to the saline dams at 8 ($p<0.01$), 24 ($p<0.001$), and 48 h ($p<0.001$) after treatment. Body temperature was not significantly affected by treatment ($p>0.05$) although a main effect of time was observed ($F_{(2.28,70.72)}=8.91$, $p<0.001$) and no time by treatment

interaction ($p>0.05$) (Figure 3.1 D). CXCL1 ($t_{(16.04)}=-3.41$, $p<0.01$) and IL-6 ($t_{(27)}=-2.62$, $p<0.05$) were significantly elevated in the polyI:C-treated dams (Figure 3.1 E). Neither CXCL2 nor TNF- α were affected by maternal treatment ($p>0.05$). On PND 1, pups from polyI:C-treated dams weighed significantly less than the saline pups (Figure 3.1 F; $t_{(31)}=2.93$, $p<0.01$) but there was no difference in litter size prior to culling to a maximum of 10 (saline = 11.94 ± 3.04 ; polyI:C = 12.00 ± 3.35 ; $t_{(31)}=0.56$, $p>0.05$). Pups were weighed once per week until weaning and the pup size difference was not seen at any other date (PND1: saline = 8.34 ± 0.16 g, polyI:C = 7.48 ± 0.28 g; PND7: saline = 22.98 ± 0.45 g, polyI:C = 22.10 ± 0.38 g; PND14: saline = 41.98 ± 0.74 g, polyI:C = 41.23 ± 0.76 g; PND21: saline = 75.85 ± 1.30 , polyI:C = 75.40 ± 1.08 g) There was no significant difference in number of pups born per litter between the groups (polyI:C: 12.00 ± 0.81 , saline: 11.94 ± 0.96 ; $p>0.05$).

3.4.2 Maternal polyI:C treatment failed to significantly affect startle or PPI

Startle responses to acoustic stimuli were assessed by measuring startle alone and PPI in saline ($n=17$ litters) and polyI:C offspring ($n=17$ litters). Startle to the 120 dB pulses alone decreased during the session (main effect of Time: $F_{(1.14,36.56)}=55.18$, $p<0.001$; Figure 3.2 B) but no Treatment interaction was present. For prepulse trials with a 30 ms (short) interval, a main effect of Prepulse Intensity on PPI ($F_{(1.42,45.47)}=21.86$, $p<0.001$; Figure 3.2 C) was found with no effect of treatment. Overall, PPI was greater at 12 dB compared to 3 and 6 dB ($p<0.001$). For trials with an 80 ms (long) prepulse-pulse interval, a main effect for Prepulse Intensity was found ($F_{(1.28,34.62)}=96.19$, $p<0.001$; Figure 3.2 D) for PPI, but no effect of Treatment and no interaction. Overall, PPI increased with louder prepulses.

Figure 3.2: Effects of maternal polyI:C on acoustic startle and PPI in the male offspring.

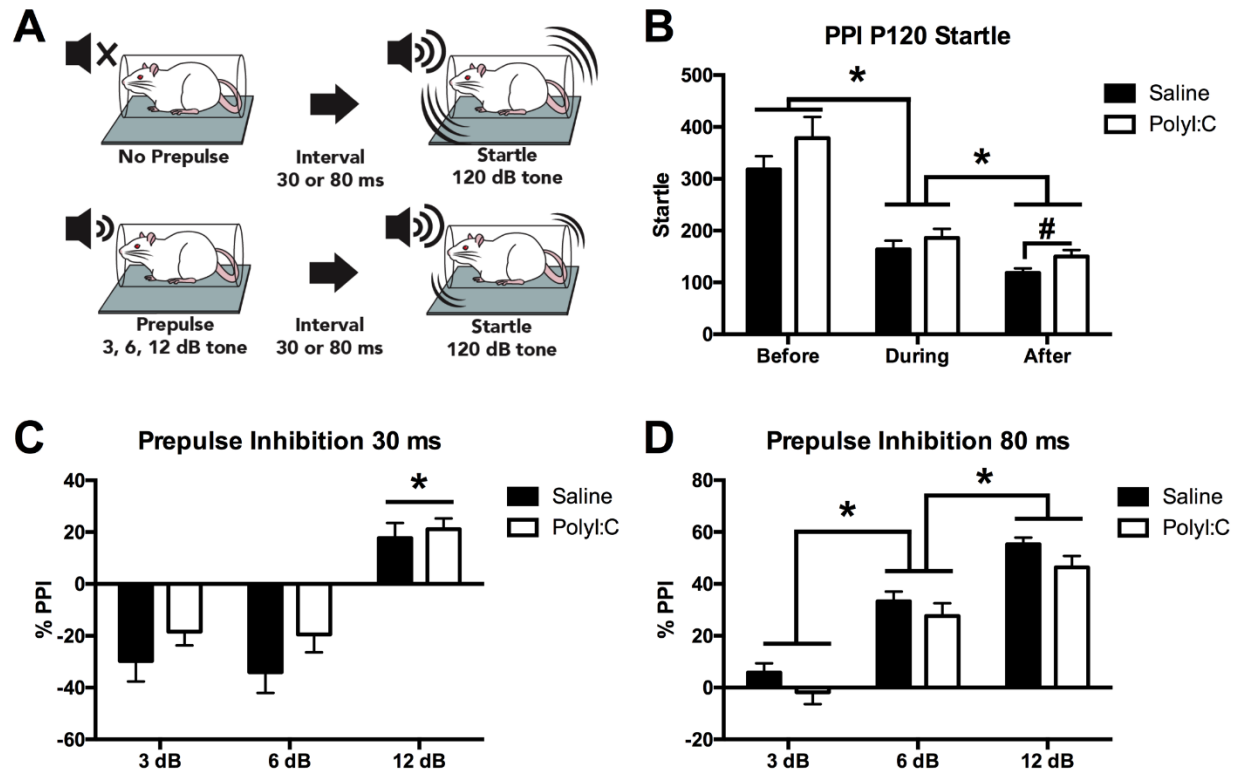


Figure 3.2: [A] Illustration of the apparatus used to measure PPI. In the top panel, no prepulse precedes the 120 dB tone and startle to the tone is high. The bottom panel highlights the typical reduction in startle response when the acoustic tone is preceded by prepulse of 3, 6, or 12 dB. [B] Offspring displayed a reduction in startle to the pulse alone trials with time, where each timepoint was significantly different than the others as indicated with asterisks. Pairwise comparisons showed higher startle reactivity in the polyI:C offspring compared to controls at the 'after' timepoint, indicated by a pound symbol (#). [C] No effect of treatment on PPI was seen for trials with a 30 ms prepulse-pulse interval. A main effect of prepulse intensity was seen with 12 dB producing greater PPI than 3 and 6 dB, indicated by asterisks. [D] No effect of treatment was seen for PPI on trials with an 80 ms prepulse-pulse interval. A main effect of prepulse intensity resulted in significantly greater PPI for trials with louder prepulses, indicated by asterisks.

3.4.3 PolyI:C offspring have reduced sociability

All offspring (n=16 saline litters, n=16 polyI:C litters) displayed a significant preference for the stranger cage (Figure 3.3 B; statistics not shown). PolyI:C offspring displayed a sociability deficit with less time exploring the stranger cage ($t_{(30)}=2.25$, $p<0.05$; Figure 3.3 C). There was no significant difference in total (stranger plus empty cage) exploration ($t_{(30)}=2.03$, $p>0.05$; Figure 3.3 C).

3.4.4 Male polyI:C-treated offspring demonstrate heightened sensitivity to MK-801

Locomotor data obtained from polyI:C (n=13 litters) and saline (n=17 litters) offspring were analyzed with repeated measures ANOVAs (Figure 3.3 E). Results revealed a main effect of Time ($F_{(2.73,76.54)}=7.67$, $p<0.001$; Figure 3.3 E). While the main effect of Treatment failed to reach significance ($F_{(1,28)}=4.14$, $p=0.051$), a significant Time by Treatment interaction was observed ($F_{(2.73,76.54)}=2.87$, $p<0.05$). Differences between the groups arose following MK-801 injections where polyI:C offspring travelled greater distances than the saline counterparts ($p\leq 0.05$).

Figure 3.3: The effects of maternal polyI:C treatment on social behaviour and MK-801 induced locomotion in male offspring.

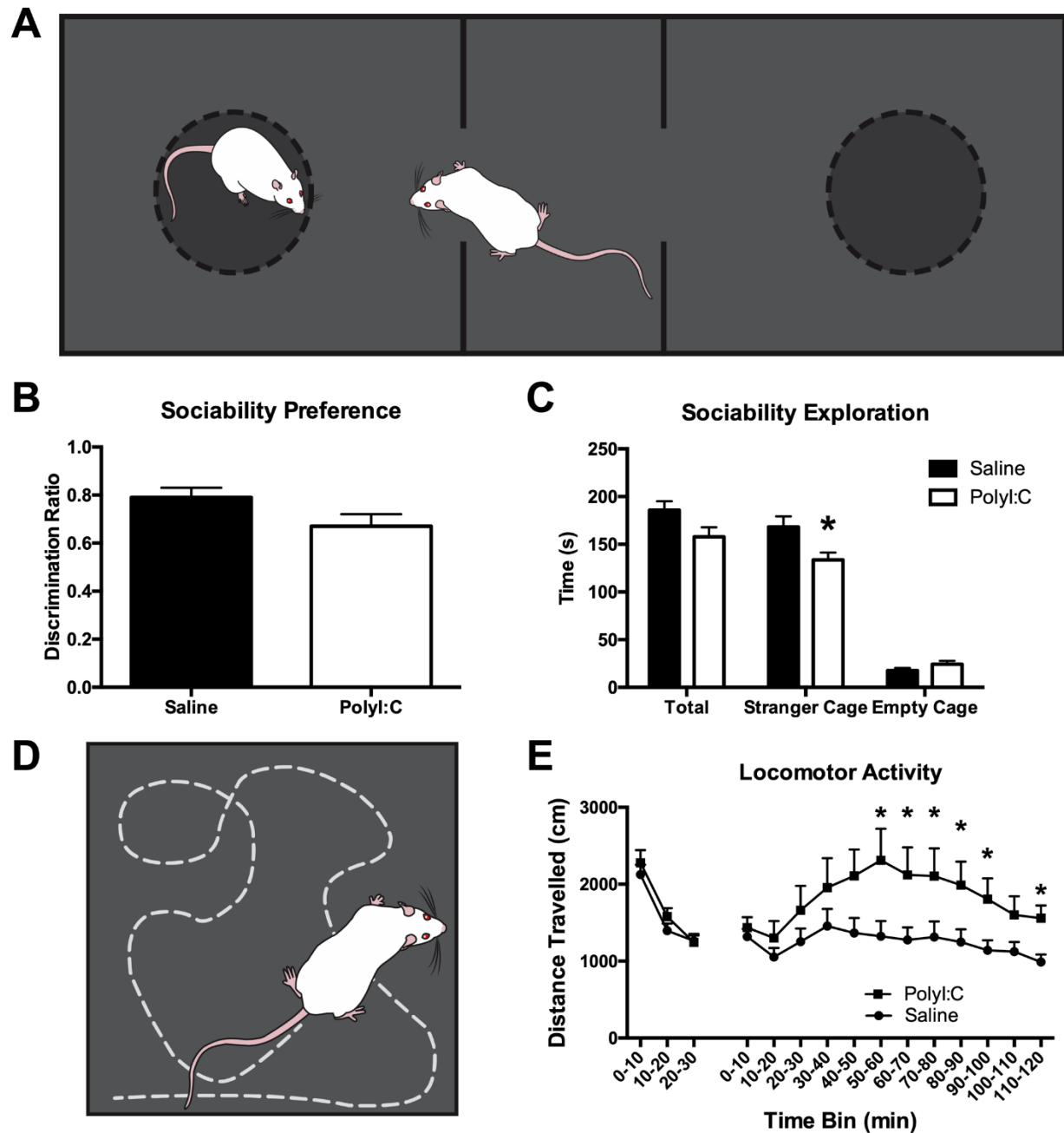


Figure 3.3: [A] Schematic of the arena used for sociability testing. A black rectangular arena is divided into three sections where the two on the ends contain identical cages and are divided by a middle section. The stranger rat is held in one of the two cages and the test rat is free to explore the entire arena (for more detail, see Materials and Methods). [B] Social interaction behaviour represented as a discrimination ratio. [C] When exploration time is divided into total exploration, stranger exploration, or empty cage exploration, polyI:C offspring spend significantly less time interacting with the stranger cage. [D] Schematic representing a rat in an open arena where locomotor activity was tracked over the course of a 30-min habituation and an additional 120-min following MK-801 injection. [E] PolyI:C offspring travelled a significantly greater distance than their saline counterparts following MK-801 treatment and the timepoints where a significant difference arose as determined by pairwise comparisons are indicated with asterisks.

3.4.5 PolyI:C offspring perform tactile object recognition memory but not crossmodal object recognition memory

All CMOR data is presented as a discrimination ratio ($\text{Exploration}_{\text{Novel}} - \text{Exploration}_{\text{Familiar}} / \text{Exploration}_{\text{Total}}$) for the first minute of the test phase ($n=15$ saline litters, 17 polyI:C litters). Both groups demonstrated significant tactile object recognition memory (saline: $t_{(14)}=13.70, p<0.001$; polyI:C $t_{(13)}=5.15, p<0.001$; Figure 3.4 B). Neither group performed above chance exploration for visual memory (saline: $t_{(14)}=1.54, p>0.05$; polyI:C $t_{(13)}=0.16, p>0.05$). In the crossmodal phase, saline offspring show significant preference for the novel object ($t_{(14)}=2.50, p<0.05$) while polyI:C do not ($t_{(16)}=0.21, p>0.05$, Figure 3.4 B). There were no differences in total object exploration times between groups in the sample and test phases (Table 3.1; statistics not shown).

3.4.6 PolyI:C-treated offspring are significantly impaired in oddity discrimination

When analyzed with a one sample t-test, saline ($n=15$ litters) offspring explored the odd object at a greater than chance level ($t_{(14)}=6.05, p<0.001$) but polyI:C offspring ($n=15$ litters) did not ($t_{(14)}=1.20, p>0.05$). When the groups were compared directly with an independent samples t-test, saline offspring spent a significantly greater % exploration with the odd object compared to polyI:C offspring ($t_{(28)}=3.92, p<0.001$; Figure 3.4 D). PolyI:C offspring spent a greater amount of time in total exploration of all objects compared to saline (saline: 67.81 ± 4.78 s; polyI:C: 84.08 ± 5.46 s; $t_{(28)}=-2.24, p<0.05$).

Figure 3.4: Maternal polyI:C treatment impaired multimodal, but not unimodal, object recognition memory in two spontaneous tasks in male offspring.

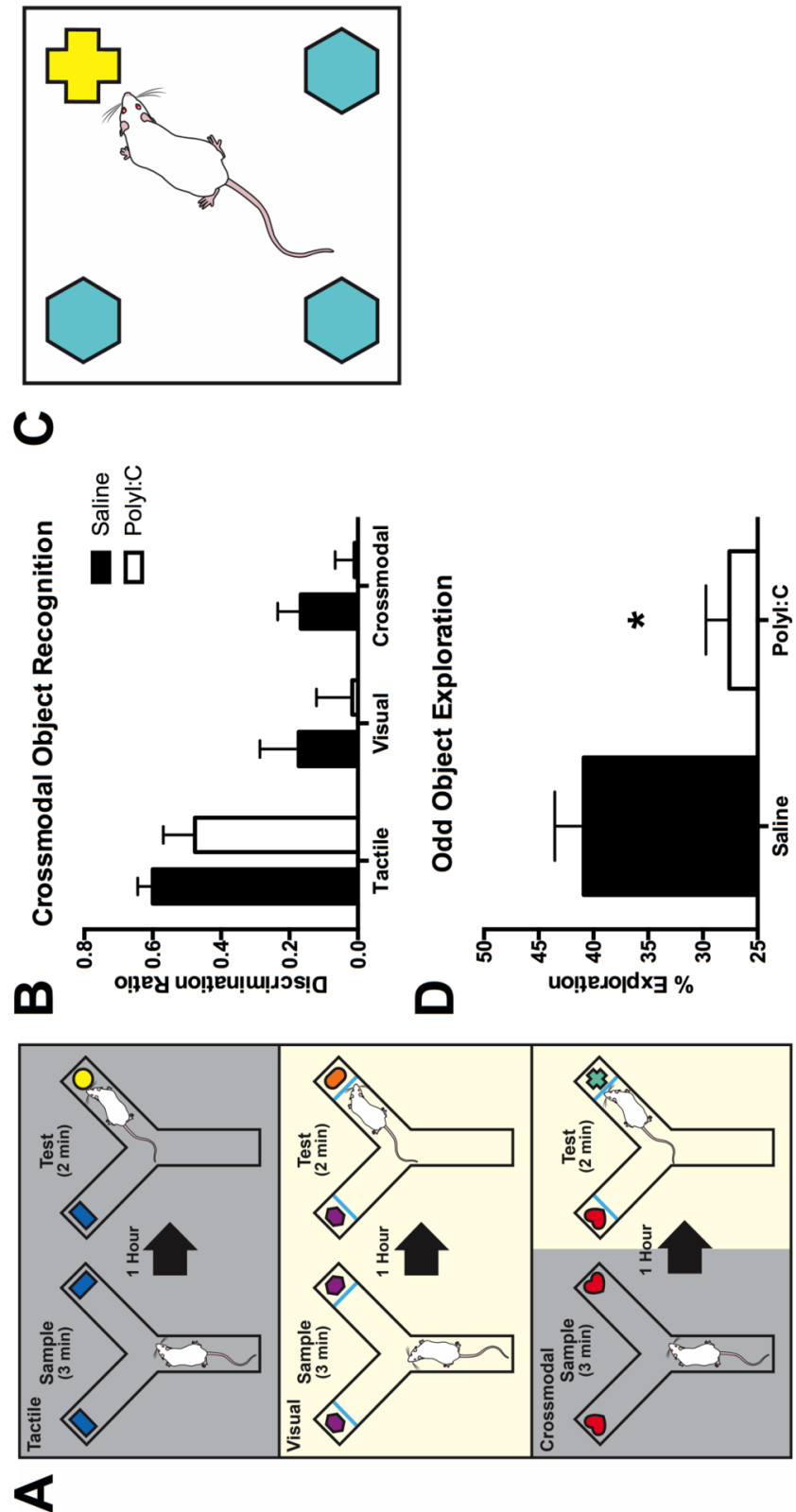


Figure 3.4: [A] Schematic of the CMOR battery including the visual (top), tactile (middle), and crossmodal (bottom) variants. Each variant contains a sample phase with two identical objects and a test phase with one familiar object from the sample phase plus one novel object (for more detail, see Materials and Methods). [B] Offspring of both treatment groups display tactile memory as indicated by a positive discrimination ratio >0 . Saline and polyI:C rats failed to perform above chance at visual memory while only saline rats displayed crossmodal memory. [C] Schematic of the oddity arena and object layout. A white square arena contained three identical objects and one odd object. Rats were allowed to explore the arena for 5 min following three habituation sessions (for more detail, see Materials and Methods). [D] Spontaneous oddity preference was significantly impaired in the polyI:C offspring.

Table 3.1: Summary of CMOR task object exploration times in male offspring.

Treatment	Task Phase	Tactile	Visual	Crossmodal
Saline	Sample	44.97 ± 3.42	6.29 ± 0.45	44.68 ± 2.73
	Test	21.40 ± 1.52	3.40 ± 0.33	3.65 ± 0.55
PolyI:C	Sample	46.29 ± 2.36	7.10 ± 0.60	41.91 ± 2.67
	Test	19.64 ± 1.42	3.05 ± 0.40	3.10 ± 0.27

Table 3.1: Total duration of object exploration during the sample and test phases for each variation of the CMOR task (mean ± SEM) for the adult offspring of dams treated with saline-or polyI:C on GD15 of pregnancy. No significant differences were seen in any groups during the sample or test phases.

3.4.7 PolyI:C offspring have contrasting alterations in behavioural flexibility tasks

Saline (n=16 litters) and polyI:C (n=16 litters) offspring acquired the visual cue stage of OSST at the same rate (saline: 65.58 ± 10.93 , polyI:C: 70.00 ± 12.20 , $p > 0.05$). The polyI:C offspring had significantly facilitated set shifting performance as indicated by fewer trials required to reach criterion ($t_{(30)} = 2.73$, $p < 0.05$; Figure 3.5 B) and fewer total errors ($t_{(30)} = 3.03$, $p < 0.01$; Figure 3.5C). Error breakdown revealed notable differences in perseverative errors with polyI:C offspring making marginally fewer ($t_{(30)} = 1.90$, $p = 0.07$; Figure 3.5 C), as well as a significant reduction in regressive errors $t_{(30)} = 2.04$, $p = 0.05$, but no difference in never reinforced errors ($t_{(30)} = 1.85$, $p > 0.05$). There were no differences in reversal learning, or other parameters of the task, including the 20 reminder trials for visual cue discrimination included on the first day of set-shifting (additional statistics not shown).

In the touchscreen PD/RL task, both groups learned the visual pairwise discrimination rule at the same rate with no differences in the number of days to criterion (DTC), total number of trials to criterion, total number of correction trials completed, or total number of errors made (statistics not shown). During reversal learning the polyI:C rats (n=17 litters) required significantly more DTC than the saline offspring (n=16 litters; $t_{(31)} = -2.19$, $p < 0.05$; Figure 3.5 C). No differences were seen for other measures. Reversal learning was then divided into early reversal (ER) which included all sessions before each rat achieved 50% correct during a single session, and late reversal (LR) which included all sessions afterwards (Bryce and Howland, 2015). While there were no differences in ER, the polyI:C offspring required more DTC than the saline offspring in LR ($t_{(31)} = -2.68$, $p < 0.05$; Figure 3.5 E), required more TTC ($t_{(31)} = -2.14$, $p < 0.05$; Figure 3.5 F), completed more correction trials ($t_{(31)} = -2.04$, $p = 0.05$), and made more errors ($t_{(31)} = -2.38$, $p < 0.05$) than the saline offspring.

Figure 3.5: Maternal polyI:C treatment facilitates set-shifting performance in an operant lever task and impairs reversal learning in an operant touchscreen task in male offspring.

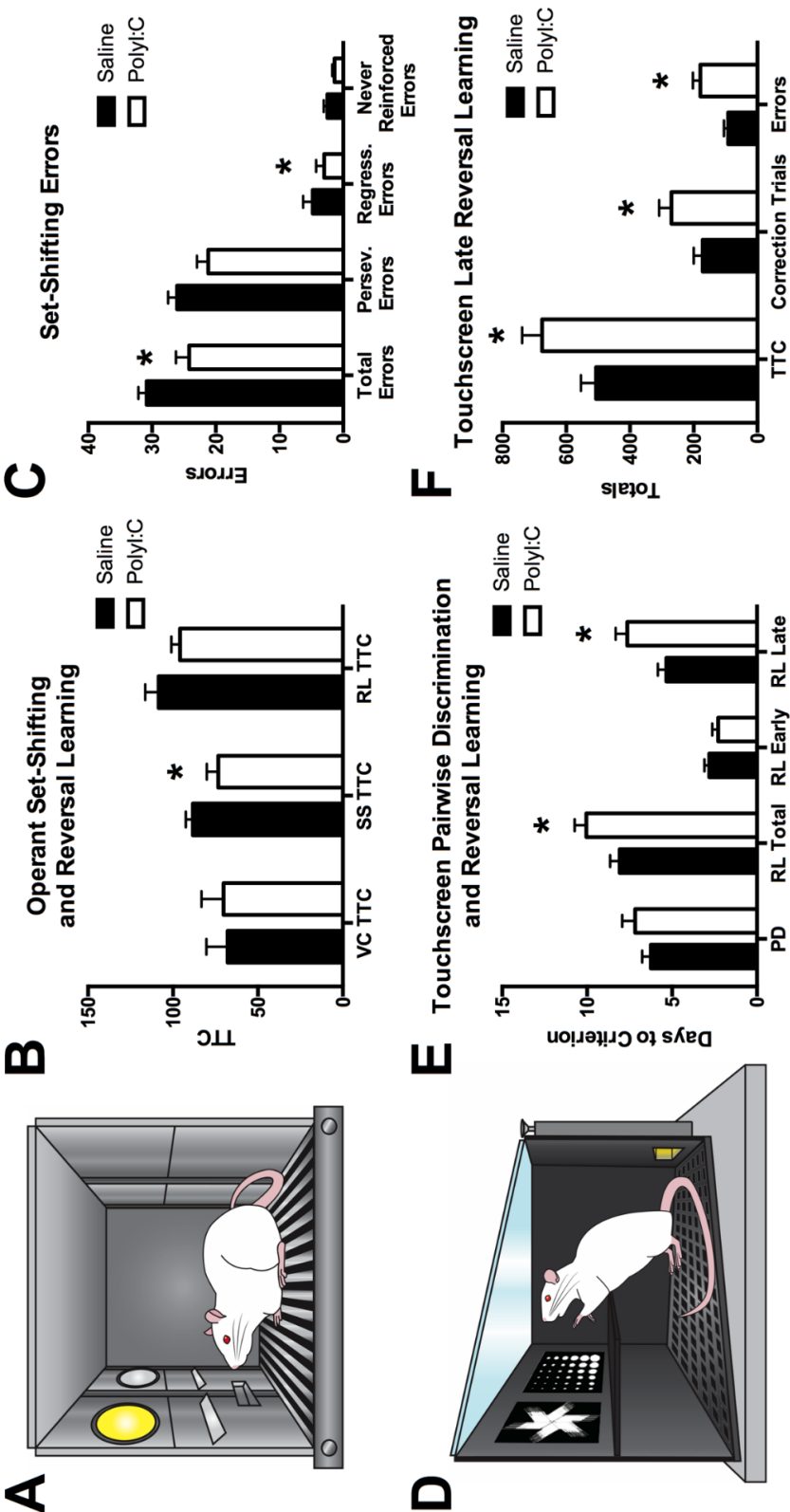


Figure 3.5: [A] Illustration of the operant chamber with lights (visual stimuli) and levers used in OSST. [B] PolyI:C and saline offspring performed visual cue learning at the same rate in OSST. PolyI:C offspring were significantly facilitated at set-shifting indicated by the fewer TTC required. The subsequent reversal learning was unaffected by maternal treatment. [C] PolyI:C offspring made fewer perseverative errors and fewer regressive errors during set-shifting compared to control rats. [D] Illustration of the touchscreen chamber and stimuli used in the PD/RL task. [E] Bar graphs displaying the number of days required to complete each stage of the PD/RL task. Saline and polyI:C offspring learned visual paired discrimination at equal rates whereas touchscreen reversal learning (RL) required significantly more days of training for polyI:C rats to reach criterion. When divided into early and late RL, no differences were seen in the early stage while polyI:C rats were significantly impaired in the late stage. [F] Comparisons of the total number of trials completed, correction trials completed, and errors made during the late RL phase showed that the polyI:C rats were impaired on all of these measures.

3.4.8 Correlations between maternal cytokines, indicators of maternal sickness, and offspring phenotypes

Maternal serum concentrations of CXCL1 were related to acute weight changes in dams treated with polyI:C at 8 h post injection ($r_{(17)}=-0.51, p<0.05$), but the effect was not robust when corrected for multiple comparisons (B-H $p>0.05$); see Table 3.2). No relationships were seen between CXCL1 levels and weight loss at 24 ($r_{(17)}=-0.22, p>0.05$) or 48 hrs ($r_{(17)}=-0.23, p>0.05$, see Figure 3.6) after injection, indicating greater weight loss in dams with the highest elevations of CXCL1. No weight-CXCL1 correlations were seen in the saline rats.

Change in maternal body temperature at 8 h post treatment was related to neonatal pup mass in polyI:C-treated rats ($r_{(17)}=0.51, p<0.05$) indicating a decrease in dam body temperature was related to the delivery of smaller pups; however this effect was not robust following adjustment for multiple comparisons (B-H $p>0.05$); see Table 3.2). No relationship was seen in saline rats. Dam weight change 48 h after polyI:C treatment was positively correlated with pup size at birth ($r_{(17)}=0.60, p<0.05$; Figure 3.6), indicating a relationship between greater sustained weight loss in the dams and the delivery of smaller pups which was robust to the multiple comparison adjustment (B-H $p<0.05$); see Table 3.2). No relationship was found in the saline group. Pup mass at birth was then negatively correlated with oddity discrimination performance (percent odd object exploration) in the polyI:C offspring only ($r_{(15)}=-0.59, p<0.05$), interestingly showing smaller pups at birth had greater preference for the odd object despite reduced oddity preference as a group, although this effect is not seen following adjustment for multiple comparisons (B-H $p>0.05$); see Table 3.2). Oddity preference was also not correlated to pup size in saline offspring. Other behaviour effects were examined for relationships to sickness effects and maternal serum cytokines but no other relationships were found (Representative results presented in Figure 3.6 A; Bivariate correlation data of distance travelled by polyI:C offspring following MK-801 administration with CXCL1: $r_{(13)}=0.34, p>0.05$; Dam weight change 8 h: $r_{(13)}=0.30, p>0.05$; Dam weight change 48 h: $r_{(13)}=0.23, p>0.05$; Pup size: $r_{(13)}=-0.07, p>0.05$; and see Table 3.2 for B-H adjusted p-values); Figure 3.6 B; Trials to criterion in late reversal learning from PD/RL task; CXCL1: $r_{(17)}=-0.30, p>0.05$; Dam weight change 8 h: $r_{(17)}=0.003, p>0.05$; Dam weight change 48 h: $r_{(17)}=-0.04, p>0.05$; Pup size: $r_{(17)}=-0.05, p>0.05$; and see Table 3.2 for B-H adjusted p-values).

Figure 3 .6: Representative plots indicating maternal cytokine levels did not correlate with male offspring behaviour outcomes.

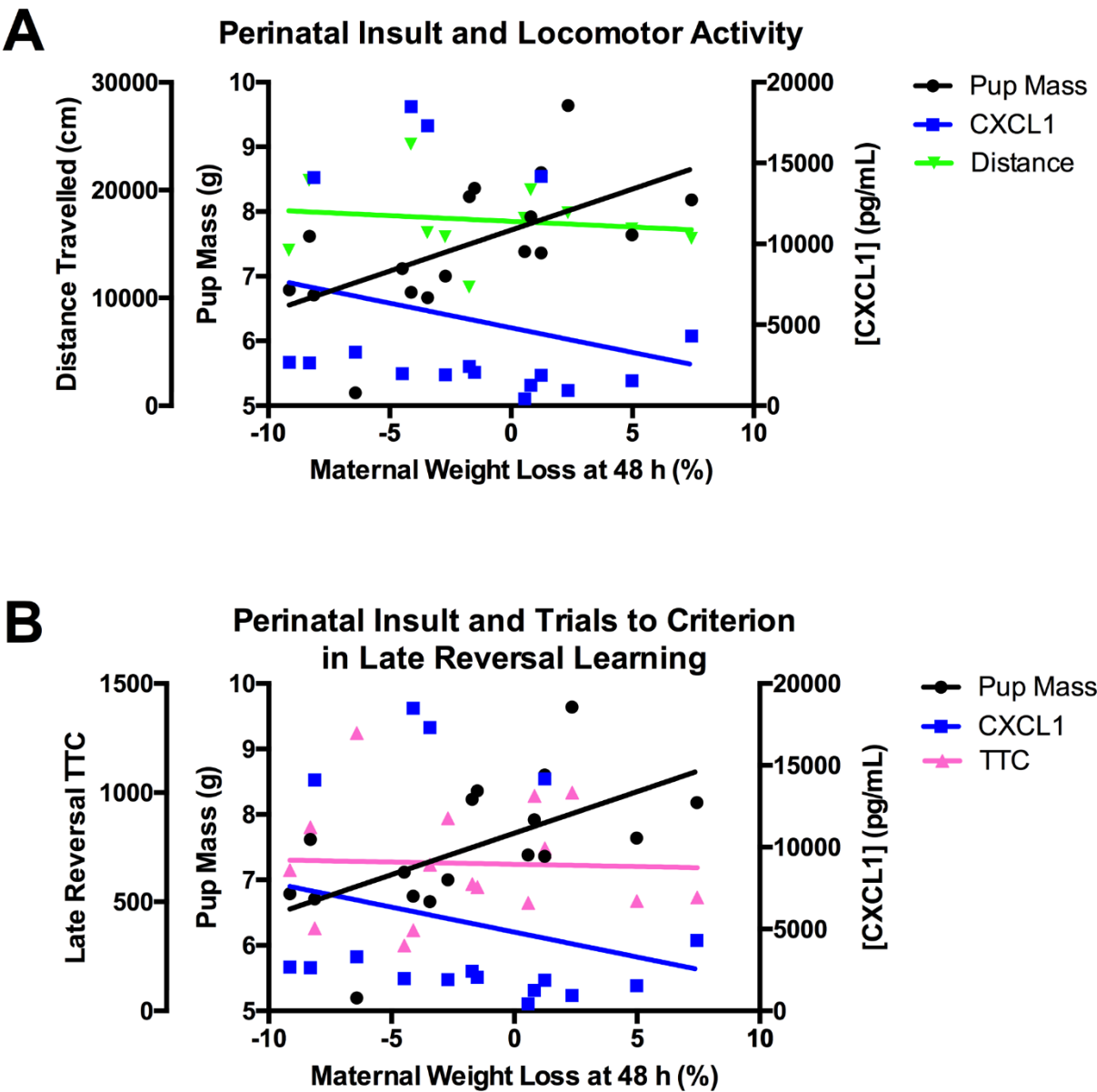


Figure 3.6: [A] Overlaid regression plots showing the relationships between acute maternal weight loss at 48 hours post polyI:C treatment and pup size, maternal CXCL1 concentration and distance travelled following MK-801 administration in polyI:C dams and offspring. Locomotor activity, used as an indicator of sensitivity to psychotomimetic compounds was increased in the polyI:C offspring, but no relationship to the measured indicators of inflammation or sickness in the pregnant dams was found. Weight change in the pregnant dams 48 h after polyI:C treatment was significantly correlated with pup size at birth ($r_{(17)}=0.60, p<0.05$). Serum CXCL1 was not related to weight change at 48 h ($r_{(17)}=-0.23, p>0.05$). Distance travelled by the offspring in the locomotor task was not related to any of these (CXCL1: $r_{(13)}=0.34, p>0.05$; Weight change 8 h: $r_{(13)}=0.30, p>0.05$; Weight change 48 h: $r_{(13)}=0.23, p>0.05$; Pup size: $r_{(13)}=-0.07, p>0.05$; see also Table 3.2). **[B]** Overlaid regression plots showing the relationships between acute maternal weight loss at 48 hours post polyI:C treatment and pup size, maternal CXCL1 concentration and trials to criterion in late reversal learning in polyI:C dams and offspring. Late reversal learning was chosen as a representative cognitive behaviour test that was altered in the polyI:C offspring yet was not related to the measured indicators of inflammation or sickness in the pregnant dams (CXCL1: $r_{(17)}=-0.30, p>0.05$; Weight change 8 h: $r_{(17)}=0.003, p>0.05$; Weight change 48 h: $r_{(17)}=-0.04, p>0.05$; Pup size: $r_{(17)}=-0.05, p>0.05$; see also Table 3.2).

3.5 Discussion

This study describes the acute effects of IV administration of polyI:C to pregnant rats and relationships between individual maternal serum cytokine concentrations and behavioural outcomes in the adult offspring. PolyI:C administration elevated maternal serum concentrations of CXCL1 and IL-6, and caused weight loss. We did not observe an effect of polyI:C treatment on maternal body temperature at 8, 24, or 48 h after treatment, in accordance with several studies (Howland et al., 2012; Sangha et al., 2014; Ballendine et al., 2015), but not others (Zhang et al., 2012). Pups of polyI:C-treated dams weighed less than controls on PND1. In young adulthood, offspring behaviours were assessed using a behaviour battery related to symptoms of psychopathology. PolyI:C offspring demonstrated behavioural changes in most tasks and serum cytokine levels in the pregnant dams correlated with weight loss, and bivariate correlations link this with offspring birth weight, however these effects appear to lack strong relationships with long-term offspring behaviour effects.

One potential limitation of our results is the use of timed pregnant rats shipped to our facility on GD7. The effects of shipment during pregnancy on the dams and unborn offspring are a valid concern (Stewart and Kolb, 1988; Ogawa et al., 2007; Kuwagata et al., 2009; Meyer et al., 2009; Moriyama et al., 2013); nonetheless, the behavioural effects in the present cohort are comparable to those from in-house bred rat offspring, particularly on MK-801 induced locomotor behaviour in the males (Zuckerman and Weiner, 2005; Vorhees et al., 2012). Timed-pregnant dams have been used in numerous similar studies that examine the roles of adverse gestational conditions and long-term effects on the offspring and constitute valuable contributions to the literature (Lodge and Grace, 2001; Du and Grace, 2013, 2016a; Van den Eynde et al., 2014)

3.5.1 Effects of polyI:C on pregnant dams and neonatal offspring

The double-stranded polyI:C molecule is recognized by the innate immune system in a similar manner as dsRNA via TLR3. Subsequent nuclear factor κ B-dependent signaling is best known for the induction of interferons; however, elevated serum concentrations of other immune proteins including IL-6, CXCL1, TNF- α and IL-1 β have been reported (Alexopoulou et al., 2001; Ballendine et al., 2015). Our results corroborate previous research with significant

elevations of IL-6 and CXCL1 in maternal serum 3 h post polyI:C injection (Ballendine et al., 2015). A discrepancy exists in the effects of polyI:C on TNF- α as previous research reports Long Evans rats, as well as Wistar rats that lost weight post treatment, showed elevated serum TNF- α (Missault et al., 2014; Ballendine et al., 2015). However, the polyI:C-treated Sprague Dawley dams did not have significantly elevated TNF- α levels in the present cohort. Elevated TNF- α is a well-established consequence of systemic inflammation when induced by polyI:C or other means such as LPS (Patterson, 2009; Mattei et al., 2014; Missault et al., 2014; Ballendine et al., 2015), although observance appears to depend on timing. A previous study demonstrated elevated TNF- α 2 h post LPS treatment which returned to control levels by 4 h. Both 3 and 6 h time points have been used by other groups, and show mixed results (Smith et al., 2007; Cui et al., 2009; Missault et al., 2014; Ballendine et al., 2015). Given these variable results and the necessity of using a single time point in the present study, we cannot be certain whether TNF- α levels reached significant elevation at any time point in the polyI:C treated dams. Weight loss following polyI:C treatment is robust in rats (Wolff and Bilkey, 2010; Piontkewitz et al., 2011b; Howland et al., 2012; Zhang et al., 2012; Missault et al., 2014; Sangha et al., 2014; Ballendine et al., 2015; Vorhees et al., 2015), consistent with established rodent sickness behaviours (Cunningham et al., 2007; Palin et al., 2009). We found that higher CXCL1 levels may be related to increased weight loss 8 h after polyI:C treatment although a greater sample size is needed to reduce the risk of type 1 error.

Maternal polyI:C-treated dams delivered pups with significantly lower body weight on PND1 compared to controls. While previous research in Long Evans rats did not show this effect (Howland et al., 2012; Ballendine et al., 2015), low birth weight in humans is a risk factor for the development of psychopathologies including schizophrenia, affective psychosis, autism spectrum disorders, attention deficit/hyperactivity disorders, and impulsivity with conduct disorders (Jones et al., 1998; Moilanen et al., 2010; Moore et al., 2012; Grissom et al., 2014; Laurens et al., 2015; Van Lieshout et al., 2015; Mathewson et al., 2017). Increased maternal IL-6 during pregnancy is associated with lower birth weights in humans (Atta et al., 2016) and our correlation data revealed a robust relationship between sustained maternal weight loss (48 h) and lower offspring birth weight in a rat model as well.

3.5.2 PolyI:C-treated offspring have behaviour abnormalities associated with psychopathology

PPI is commonly used to assess sensorimotor gating which is altered in many psychiatric conditions and can be disrupted through the administration of DAergic and glutamatergic agonists. Earlier studies that assessed PPI in models of MIA showed PPI disruptions (reviewed in Meyer et al., 2009), however more recent work has failed to reproduce this effect (Missault et al., 2014; Van den Eynde et al., 2014; Vorhees et al., 2015). In agreement with several recent papers, our data showed no appreciable effect of MIA on PPI in the offspring.

PolyI:C offspring showed heightened sensitivity to MK-801. This finding replicates previous studies which report hyperlocomotion following MK-801 (Zuckerman and Weiner, 2005; Howland et al., 2012; Giovanoli et al., 2013) although some other studies have observed hypolocomotion (Vorhees et al., 2012, 2015; Missault et al., 2014). As the administration of NMDA receptor antagonists increases striatal DA efflux, exaggerated locomotor activity following MK-801 may relate to the positive symptoms of the disorder and increased striatal DA in schizophrenia patients (Usun et al., 2013; Laruelle, 2014).

Abnormal social functioning is seen in many neurological conditions (Kennedy and Adolphs, 2012) and we demonstrated a sociability deficit in adult polyI:C-treated offspring. Illnesses with a neurodevelopmental component like autism and schizophrenia have social deficits as a central feature, which is fitting with our finding that an early developmental insult impacted this behaviour domain. Normal social functioning is dependent on neurodevelopmental processes as shown by the particularly deleterious effects of early life insult (Lee and Green, 2016). Early life deletion of the NR1 subunit, but not post adolescent deletion in mice results in impaired social preference and early childhood PFC damage in humans is associated with impaired sociability (Anderson et al., 2000). The network of regions with known roles in social cognition and social behaviours is diffuse, and include the PFC as well as the temporal lobe and amygdala, involved in facial and emotional recognition respectively, both of which are known to be altered in autism and are believed to contribute to the characteristic social deficits (Kennedy and Adolphs, 2012). The developmental effects of maternal inflammation are broad, and the impairments seen in our study could be related to the impact of developmental immune insult in one or several of these areas. NMDA receptor hypofunction induced by pharmacologic, genetic,

and optogenetic means in mice disrupts preference for a stranger conspecific, and there is prior evidence for NMDA receptor disruption in MIA offspring as well as structural changes in the brain regions required for typical social proficiency, particularly the PFC (Samuelsson et al., 2006; Piontkewitz et al., 2011a; Lee and Green, 2016).

3.5.3 PolyI:C administration during pregnancy results in altered performance in cognitive behaviour tasks in the adult offspring

The CMOR task assesses multisensory integration and the ability to form complex, multimodal representations of stimuli (Cloke et al., 2015; Jacklin et al., 2016). Previous research showed rats are capable of tactile-visual crossmodal memory indicated by greater visual exploration of a novel object compared to an object previously experienced tactilely (Winters and Reid, 2010). The role of the perirhinal cortex (PRh) and posterior parietal cortex (PPC) in visual and tactile recognition memory respectively is well known (Zhou and Fuster, 1997; Buckley and Gaffan, 1998; Murray and Richmond, 2001; Buckley, 2005; Albasser et al., 2011), although lesion studies have shown bilateral ablation of the PFC (medial PFC and OFC) results in selective disruption of CMOR while leaving the control visual-visual and tactile-tactile memory tasks intact. More precise lesions reveal the necessary role of the OFC in mnemonically demanding situations (Winters and Reid, 2010; Reid et al., 2014; Cloke et al., 2015). We have previously demonstrated polyI:C offspring show specific deficits in CMOR in polyI:C offspring male Long-Evans rats (Author references to be included following peer review). The present study with Sprague-Dawley rats found a deficit in both visual and crossmodal domains of the task, effects suggesting maternal inflammation altered function of the OFC and PRh.

Oddity discrimination is a recently developed task that may assess the function of the ventral visual stream (VVS) and temporal lobe memory system in a spontaneous exploration paradigm. The Perceptual-Mnemonic/Feature-Conjunction neural network model suggests the PRh is situated at the most distal end of the VVS where it supports complex representations of an object comprised of simpler visual components which are hierarchically maintained in the more caudal regions of the VVS (Murray and Bussey, 1999; Bussey and Saksida, 2005; Bussey et al., 2005; Bartko et al., 2007a). Visual learning and memory was highlighted as one of seven cognitive domains impaired in schizophrenia (Young et al., 2009), and inactivation of the PRh

impairs oddity discrimination in rodents (Bartko et al., 2007a), while structural abnormalities in the temporal lobe are seen in patients with schizophrenia as well as animal models including MIA (Piontkewitz et al., 2012). The oddity discrimination task reveals that polyI:C-treated offspring are impaired at performing a non-mnemonic visual discrimination with no delay, suggesting altered function of the VVS which feeds into the temporal lobe memory system, including the PRh. We observed a possible relationship where smaller pups had greater oddity preference, but this was not robust when controlled for multiple comparisons. A suggestion for resilience could be that small offspring received greater maternal care. A previous study [Author reference] found no changes to maternal behaviour in dams treated with polyI:C, however that cohort did not show a difference in pup size which limits the ability to make direct comparisons.

Cognitive flexibility enables the direction of appropriate behavioural responses following changing environmental demands. Set-shifting and reversal learning are related, yet dissociable behaviours used to measure cognitive flexibility (Floresco et al., 2009). In rodents, the operant set-shifting and reversal learning task (OSST) has been heavily studied and assesses both of these measures following the learning of a simple rule. Set-shifting requires inhibition of the initial behaviour response pattern and adoption of a new strategy (extradimensional shift, EDS), and subsequently reversal learning requires performing the opposite behaviour within the same dimension as the previous stage (intradimensional shift, IDS). In the paradigm used for this study, rats learned to press a lever indicated by an illuminated light (visual cue), strategy set-shift to press either the left or right lever regardless of visual stimuli (spatial cue), and finally reverse their behaviour to press the opposite lever (Floresco et al., 2008, 2009; Thai et al., 2013; Ballendine et al., 2015; Brady and Floresco, 2015). We used a similar procedure in touchscreen operant chambers where rats learn to select one of two visual stimuli on a screen (pairwise discrimination) followed by reversal which required selection of the opposite stimuli (Bryce and Howland, 2015). Set-shifting behaviour is known to depend on the mPFC while reversal is orbito-frontal dependent (Floresco et al., 2009).

We observed a facilitation of performance in the set-shifting portion of OSST, characterized by less perseveration, less regression, and more rapid acquisition of a novel behaviour strategy with no significant effect on RL. These results are inconsistent with some prior studies (Zuckerman and Weiner, 2005; Savanthrapadian et al., 2013). Impaired strategy set-

shifting has been reported in the offspring of polyI:C-treated Long Evans rats (Zhang et al., 2012; Ballendine et al., 2015). Another previous study however, reports subchronic ketamine administration prior to OSST resulted in reduced perseveration during set-shifting, however this was accompanied with impaired learning of the initial discrimination and impaired reversal learning with increased perseveration when the present study reports no effect on initial cue learning or RL in this task paradigm (Floresco et al., 2009). In further contrast, polyI:C offspring were impaired in late reversal in the touchscreen PD/RL task. The specificity of impairment to late reversal suggests the polyI:C-treated offspring have difficulty identifying the new rule once they have ceased perseverating during early reversal learning, and impaired reversal learning in polyI:C offspring has been previously reported in spatial memory dependent tasks (Savanthrapadian et al., 2013, but see Zuckerman and Weiner, 2005). The role of DA in cognitive flexibility and behaviour shifting is complex, where previous work suggests PFC DA depletion can facilitate EDS, yet blockade of D1 and D2 in the mPFC is known to impair with a specific increase in perseveration (Roberts et al., 1994; Ragozzino, 2002b; Floresco et al., 2006). The complex role of DA in cognitive flexibility is seen where the administration of AMPH in rats results in impairments in set shifting, or impaired reversal learning with no effect on set shifting when compared across several studies (Weiner and Feldon, 1986; Russig et al., 2003; Fletcher et al., 2005). Abnormalities in DA transmission have been reported in MIA models including increased DA turnover and increased D2 binding (Ozawa et al., 2006). These results generally support that MIA results in offspring abnormalities in PFC DA.

Table 3.2: Grouped bivariate correlation data.

<i>Grouping</i>	<i>Correlation</i>	<i>Treatment</i>	<i>Uncorrected p-value</i>	<i>B-H Adjusted p-value</i>
<i>Maternal- Maternal</i>	CXCL1-Dam Weight 8h	Saline	0.28	0.40
		PolyI:C	0.04*	0.19
	CXCL1-Dam Weight 24h	Saline	0.063	0.19
		PolyI:C	0.40	0.40
	CXCL1-Dam Weight 48h	Saline	0.30	0.40
		PolyI:C	0.38	0.40
<i>Maternal- Neonate</i>	Dam Temp 8 h-Pup Mass PND1	Saline	0.36	0.36
		PolyI:C	0.04*	0.07
	Dam Weight Loss 48h-Pup Mass PND1	Saline	0.35	0.36
		PolyI:C	0.01*	0.04*
<i>Neonate- Behaviour</i>	Pup Mass-% Oddity	Saline	0.16	0.32
	Preference	PolyI:C	0.02*	0.08
	Pup Mass-Locomotor	PolyI:C	0.81	0.81
	Pup Mass-TTC LR	PolyI:C	0.25	0.33
<i>Maternal- Behaviour</i>	Dam Weight 8h-Locomotor	PoyI:C	0.32	0.64
	Dam Weight 48h- Locomotor	PolyI:C	0.46	0.69
	Dam Weight 8h-TTC LR	PolyI:C	0.99	0.99
	Dam Weight 8h-TTC LR	PolyI:C	0.89	0.99
	CXCL1-Locomotor	PolyI:C	0.26	0.64
	CXCL1-TTC LR	PolyI:C	0.25	0.64

Note: TTC RL – Trials to Criterion Late Reversal; Locomotor = distance travelled following MK-801 administration in the locomotor activity task

Table 3.2: Summary of the notable correlations between maternal and offspring measures taken in the present study. Correlations were grouped into 4 families to apply the B-H adjustment for multiple comparisons. The unadjusted p-values ($\alpha=.05$) are presented alongside the False Discovery Rate (Benjamini-Hochburg) corrected p-values. Significance in each column is indicated with an asterisk (*).

3.5.4 Maternal serum cytokine levels following polyI:C treatment are not strongly related to offspring behaviour outcomes

Prospective human studies suggest elevated cytokines in the maternal serum during pregnancy contribute to an increased risk of developing schizophrenia (Brown et al., 2004a, 2004b; Brown, 2012, 2006; Ellman et al., 2010). In one sample, human IL-8 (and not IL-6, TNF- α , or IL-1 β) was elevated in the serum of pregnant women whose offspring went on to develop schizophrenia (Brown et al., 2004) and correlated with brain changes in the offspring (Ellman et al., 2010). In rodent models of MIA, maternal IL-6 released in response to MIA is necessary for schizophrenia-like symptoms in the mouse model (Smith et al., 2007) and administration of IL-6 alone to pregnant rats is sufficient to cause behavioural changes in the offspring (Samuelsson et al., 2006). Ballendine and colleagues (2015) also used an antagonist (G31P) for CXCL1 receptors (CXCR1/R2) in an attempt to block the behavioural effects of polyI:C in Long Evans rats with mixed results. We sought to determine the relationship between individual maternal serum cytokine levels following an inflammatory insult and offspring phenotype to better understand the mechanisms underlying the behavioural effects of MIA (Meyer, 2014). Cytokines play diverse roles in brain development and affect functions such as induction and renewal of neuroepithelial cells which form scaffolding for migrating neurons, and neuronal migration (see Deverman and Patterson, 2009; Stolp, 2013 for comprehensive reviews). IL-6 influences fate switching and cell differentiation in development while IL-6- and CXCL1-related signaling influences brain development by regulating neurogenesis, maturation, and survival (Ip, 1998; Nakashima et al., 1999; Cho and Miller, 2002; Ragozzino, 2002a; Gregg and Weiss, 2005; Deverman and Patterson, 2009; Arrode-Brusés and Brusés, 2012; Garay et al., 2013; Stolp, 2013).

In our study, the offspring of rats that experienced an immune challenge in pregnancy displayed a variety of behaviour abnormalities that are associated with psychiatric and neurological disease, yet individual maternal serum cytokines measured acutely following treatment with polyI:C were not strongly related to these effects. The altered behaviours are known to depend largely on overlapping brain regions including the mPFC, OFC, striatum, amygdala, perirhinal cortex and post parietal cortex. Positive symptoms are often attributed to hyperactivity of the mesolimbic DA system while hypoactivity of mesocortical DA is linked to

negative and cognitive symptoms (Abi-Dargham and Moore, 2003; Laruelle et al., 2003; Winterer and Weinberger, 2004; Guillin et al., 2007; Jarskog et al., 2007; Meyer and Feldon, 2009). Mechanisms by which LPS- and polyI:C-induced inflammation during pregnancy may influence development have been explored, and inflammatory events can lead to increased TH in the NAc of offspring, elevated DA, and reduced DA receptors in the PFC (Bacopoulos and Bhatnagar, 1977; Borrell et al., 2002; Romero et al., 2007; Meyer et al., 2008a, 2008b; Meyer and Feldon, 2009). These DA-related changes may be associated with increased IL-6 in development (Okubo et al., 1997a, 1997b; Meyer et al., 2008b, 2008a; Meyer and Feldon, 2009). Cognitive impairment is also linked to NMDA receptor signaling (Moghaddam et al., 1997; Moghaddam and Adams, 1998; Vinson and Conn, 2012). IL-6 elevations in late gestation increases NR1 expression in the adult hippocampus, although polyI:C induced maternal inflammation produced the opposite effect in the offspring (Samuelsson et al., 2006). The effort to identify individual causal inflammatory mediators may be challenging due to the diffuse effects of systemic inflammation in pregnancy. Multiple inflammatory and anti-inflammatory signaling pathways are initiated by TLR3 stimulation and the effects on neurodevelopment may still not be fully appreciated. We found significant correlations between acute serum CXCL1 and acute maternal weight loss although these failed to translate to the broad offspring behaviour abnormalities observed. Thus, our results suggest measures other than levels of individual circulating maternal inflammatory cytokines may be more informative of long-term behavioural outcomes of the offspring. For example, future experiments including multivariate analyses of an array of maternal inflammatory markers may provide more valuable predictive information about offspring behaviour.

CHAPTER 4

MATERNAL IMMUNE ACTIVATION DURING PREGNANCY ALTERS THE BEHAVIOUR PROFILE OF FEMALE OFFSPRING OF SPRAGUE-DAWLEY RATS³

4.1 Abstract

Sex differences are documented in psychiatric and neurological disorders, yet most preclinical animal research has been conducted in males only. There is a need to better understand of the nature of sex differences in brain disease in order to meet the needs of psychiatric patients. We present the behaviour profile of adult female offspring produced using a maternal immune activation model where pregnant rats receive an immune stimulant and the offspring typically show various abnormalities consistent with psychiatric illnesses such as schizophrenia and autism. The results in female offspring were compared to a previously published cohort of their male siblings (Lins et al. 2018). We examined PPI, sociability, MK-801 induced locomotor activity, CMOR, and oddity discrimination; behaviours relevant to the positive, negative, and cognitive symptoms of schizophrenia. No between-treatment differences in PPI or locomotor activity were noted. Tactile memory was observed in the control and treated female offspring, visual recognition memory was deficient in the polyI:C offspring only, and both groups lacked crossmodal recognition. PolyI:C offspring were impaired in oddity preference and had reduced preference for a stranger conspecific in a sociability assay. Systemic maternal CXCL1, IL-6, and TNF- α levels at 3 h post polyI:C treatment were determined, but no relationship was found between these cytokines and the behaviour seen in the adult female offspring. Overall, female offspring of polyI:C-treated dams display an array of behaviour

³ Lins BR, Marks WN, Zabder NK, Greba Q, Howland JG (2019) Maternal immune activation during pregnancy alters the behaviour profile of female offspring of Sprague Dawley rats. *eNeuro*.0437-18.2019

Lins organized and led the experiments, collected and analyzed data, and co-wrote the manuscript; Marks, Zabder, and Greba collected behaviour data and contributed to data scoring and data analysis; Howland supervised the experiments and co-wrote the manuscript. This manuscript is peer-reviewed and published.

abnormalities relevant to psychiatric illnesses such as schizophrenia similar to those previously reported in male rats.

4.1.1 Significance statement

Sex differences are documented in mental illness and include differences in disease prevalence, symptom presentation, and response to treatment. Despite this, the majority of animal research has been conducted in males only. This study demonstrates the effects of maternal inflammation in pregnancy on long-term behaviour outcomes in female offspring, revealing a behaviour profile similar to male counterparts. We use a uniquely broad behaviour testing battery to show that female offspring from inflammation-exposed pregnancies display an array of abnormal behaviours related to symptom domains of schizophrenia, similar to their male littermates. Maternal cytokine concentrations did not correlate with the severity of these behaviour changes suggesting other factors may better indicate long-term disease risk in the offspring.

4.2 Introduction

Adverse events in utero and early life are linked to the development of psychiatric illness. Inflammation during pregnancy is associated with increased risk of psychiatric illnesses including autism, schizophrenia, and major depression in the offspring (Patterson, 2011; Jiang et al., 2016; Brown and Meyer, 2018; Gustafsson et al., 2018). The relationship between inflammation and psychopathology is often studied with models of maternal immune activation (MIA) where an immune stimulant such as polyI:C is administered to pregnant rodents (Piontkewitz et al., 2012; Brown and Meyer, 2018). Offspring of treated dams display behavioural and neuropathological profiles consistent with psychiatric illness in humans (Brown and Meyer, 2018). The majority of MIA studies focus on the male offspring or lack consideration of sex as a biological variable despite policies by the National Institute of Health (NIH) and other grant funding agencies that require the examination of sex as a factor in biomedical research (Clayton and Collins, 2014; Coiro and Pollak, 2019). This is particularly concerning for studies of MIA given the sex differences noted in the human psychiatric disorders

associated with MIA as a risk factor (Klein and Corwin, 2002; Arad et al., 2017; Brown and Meyer, 2018; Coiro and Pollak, 2019).

Previous studies of the effects of MIA during pregnancy in rats has resulted in extensive knowledge of behaviour effects in male offspring. Maternal treatment with polyI:C during gestation results in male offspring with reduced working memory span capacity, dysregulated fear responses, and impaired associative (object-in-place) and crossmodal memory while simple object recognition and object-location memory are largely unaffected, although impaired novel context recognition has also been reported (Wolff and Bilkey, 2010; Wolff et al., 2011; Sangha et al., 2014; Ballendine et al., 2015; Murray et al., 2017). Other studies on adult male offspring from polyI:C-treated pregnancies have found reduced levels of GAD67 in the dorsal hippocampus which appears to coincide with a loss of coherence with the mPFC and correlate with PPI deficits (Dickerson et al., 2010, 2013, 2014; Wolff and Bilkey, 2010). PPI has been studied extensively in MIA rat models, yet the results are mixed with several studies showing deficits, no effect, or indicate the timing and type of inflammatory agent determines PPI outcomes (Fortier et al., 2004, 2007, Wolff and Bilkey, 2008, 2010; Ballendine et al., 2015; Hadar et al., 2015). Other studies with offspring of both sexes or sex unspecified report mixed results on PPI as well (Howland et al., 2012; Klein et al., 2013; Van den Eynde et al., 2014; Vorhees et al., 2015). Further MIA studies including male and female rat offspring report altered behaviours such as spontaneous hypolocomotion (Van den Eynde et al., 2014), latent inhibition (Zuckerman and Weiner, 2003, 2005; Zuckerman et al., 2003), and reduced startle (Van den Eynde et al., 2014). Other studies show no change in spontaneous locomotion but reduced sensitivity to the hyperlocomotive effects of AMPH treatment (Bronson et al., 2011) or hyperlocomotion following AMPH (Zuckerman et al., 2003; Vorhees et al., 2012) or MK-801 (Zuckerman and Weiner, 2005; but see also Howland et al., 2012). In some tasks, male rat offspring of MIA dams show greater impairments in tasks such as operant conditioning-based set shifting and earlier onset of latent inhibition deficits, reflecting the earlier onset of abnormal developmental trajectories (Piontkewitz et al., 2011a; Zhang et al., 2012; Patrich et al., 2016). While male MIA rats have impaired object-in-place memory, neither control nor MIA females perform this task, possibly reflecting sex-specific differences in baseline task performance (Howland et al., 2012; Ballendine et al., 2015). In a related rat model of MIA, inflammation

induced in lactating dams, resulting in the development of distinct sex-dependent phenotypes in the suckling offspring, where the females offspring displayed a depressive phenotype and male offspring displayed a psychiatric phenotype (Arad et al., 2017). Taken together, the complicated and often conflicting results from these studies demonstrate the need for sex by treatment analyses in future MIA research (Coiro and Pollak, 2019; Kentner et al., 2019).

The present study aims to contribute to the necessary evaluation of sex effects in the MIA model by highlighting female offspring behaviour outcomes in tasks related to the symptoms of schizophrenia and analyzing these results in conjunction with previously published results in their male littermates. The male MIA offspring from the same cohort display hyperlocomotion following MK-801 administration, reduced sociability, impaired visual recognition memory, impaired oddity preference, altered set shifting, and facilitated reversal learning. Using a prospective design, we showed that these behavioural changes did not correlate with acute elevations in a selection of maternal serum cytokines collected 3 h post polyI:C treatment (Lins et al., 2018). We hypothesized that behaviour abnormalities would be less severe or absent in early adulthood in accordance with previous literature (Piontkewitz et al., 2011a; Zhang et al., 2012; Patrich et al., 2016). We also correlated behaviour of the female offspring with the acute cytokine concentrations from prospectively collected maternal blood and other measurements related to polyI:C treatment to assess relationships between acute maternal cytokine levels, other treatment effects, and offspring behaviour including an oddity discrimination task not previously examined in female rats (Lins et al., 2018).

4.3 Materials and methods

4.3.1 Animals

Timed-pregnant Sprague-Dawley rats (n=43, Charles River, Quebec) arrived at the animal holding facility on GD7. Primiparous dams were mated between 8 – 10 weeks of age and the presence of a vaginal plug considered GD1. Sires were a minimum of 10 weeks of age at time of mating and their specific breeding history (number of matings, successful pregnancies, mating design, or time between matings) was not guaranteed by the provider. Upon arrival, pregnant dams were housed individually in standard ventilated (395 x 346 x 227 mm) polypropylene cages. Food (Purina Rat Chow) and water were available ad libitum. The colony room is

temperature, but not humidity, controlled (21°C) and maintained on an automatic 12/12 h light/dark cycle with lights on at 07:00 h. Dams were undisturbed until they received treatment on GD15. Behaviour testing was conducted on adult female offspring (total n=71). All procedures were carried out during the light phase and were conducted in accordance with the Canadian Council on Animal Care guidelines for humane animal use and were approved by the University of Saskatchewan Animal Research Ethics Board.

4.3.2 Maternal treatments and blood samples

Maternal treatment followed previously established protocols in Long-Evans and Sprague Dawley rats (Howland et al., 2012; Zhang et al., 2012; Sangha et al., 2014; Lins et al., 2016; Paylor et al., 2016; Lins et al., 2018; Figure 4.1 A). Additional information is included to improve the scientific rigor of the MIA model as discussed by Kentner and colleagues (2018). These details also apply to our recent companion publication which tested male rat offspring from the same litters as described here (Lins et al. 2018).

Dam baseline weight (mean = 346.5 g) and rectal temperature were recorded on GD15 immediately prior to anesthesia with isoflurane (5% induction, 2.5% maintenance, for approximately 3 minutes) to receive a single intravenous (i.v.) tail vein injection of 0.9% saline or polyI:C (4 mg/kg, high molecular weight, InVivoGen, San Diego, CA, USA, thawed from storage at -20 °C). Dams were anesthetized a second time (as above, approximately 10 minutes) 3 h following initial treatment and a blood sample (<1.5 mL total and <6% total blood volume) was collected using a sterile catheter (BD Insyte™ Autoguard™, 24 GA 0.75 IN 0.7 x 19 mm, REF 381412) from the opposite tail vein used to inject polyI:C or saline. Warm saline was administered once following initial treatment (3 mL), and a second time after blood collection (equal in volume to the collected blood sample). Blood samples coagulated at room temperature for 1 h then centrifuged at 10,000xG for 5 min and serum was stored at -80 °C until analysis. ELISAs for CXCL1 (GRO α /KC; R&D Systems, Minneapolis, MN), CXCL2 (GRO β /MIP-2; R&D Systems), IL-6 (PeproTech, Rocky Hill, NJ), and TNF- α (PeproTech) were performed according to the manufacturer's instructions and these results are published elsewhere (Lins et al., 2018).

Dams had weight and rectal temperature measured 8, 24 and 48 h post treatment and then were undisturbed for the remainder of their pregnancy. Treatment was administered to n=43 dams, but 8 developed hypothermia and were euthanized within 48 h of treatment. Four additional dams experienced body temperature below 36°C but lacked additional indicators of severe sickness or suffering and these were given access to a warming pad on their home cage until their temperature returned to normal (within 24 h). 2 dams did not produce viable litters and 2 litters had no female offspring. Ultimately, offspring from a total of n=31 litters were included (n=16 polyI:C treated dams and n=15 saline treated dams, Table 4.1). On postnatal day (PND) 1, litters were weighed, sexed, and culled to a maximum of 10 (4 females where possible). Standard husbandry included cage changes twice per week with one additional cage change during PND14-21. Prior to weaning, all cage changes, feeding, and monitoring of pups was performed by a single investigator to minimize disturbances. On PND23, pups were weaned and housed in same-sex sibling groups of 2 or 3 in standard housing as previously described with a PVC tube for enrichment.

Figure 3.1: Timelines of maternal treatments and female offspring behaviour testing.

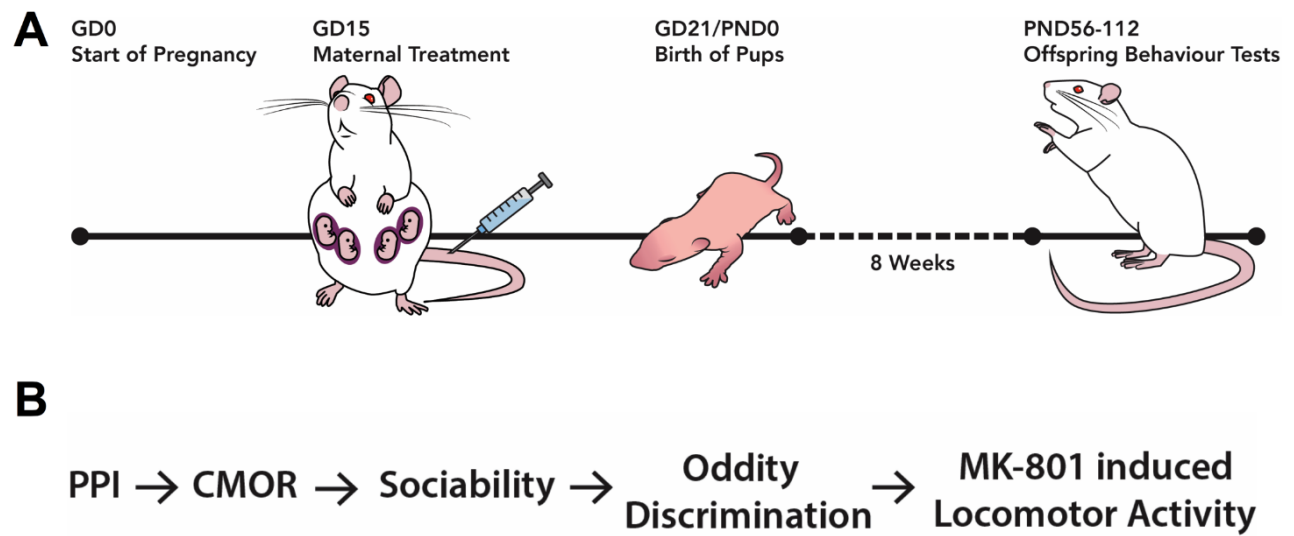


Figure 4.1: [A] Schematic detailing the time line of maternal treatment and initiation of offspring behaviour testing. Schematic has been published previously (Lins et al., 2018). [B] Flow chart depicting the order of the behaviour test battery for female offspring.

Table 4.1: Summary of dams' treatment, adverse events and litter data.

Treatment	Total Dams Treated	Dams Euthanized	Dams w/ No Litter	Litters Included	Viable Offspring
Saline	18	0	2 (+1 no ♀)	15	11.94 ± 0.76
PolyI:C	25	8	0 (+1 no ♀)	16	12.00 ± 0.81

Table 4.1: 8 dams were euthanized within 48 h of polyI:C administration because they developed low body temperature and showed sickness behaviours beyond what is acceptable as outlined in our Humane Intervention Protocol. One litter per treatment included male, but no female offspring, resulting in exclusion from the final count of litters included in this chapter. The 2 saline-treated control dams that did not produce litters showed no evidence of pregnancy. Viable offspring count per dam includes all surviving offspring of both sexes present on PND1 prior to culling to a maximum of 10, excludes dams that did not give birth, and is presented as $\mu \pm \text{SEM}$. All additional data presented on the dams only includes those that produced viable offspring.

4.3.3 Behavioural testing

Behaviour tests were conducted according to published protocols. One or two female offspring per litter were included in each test, except PPI where all available females were included (Table 4.2 a-c). To control for the inherent relationships between siblings, effects from littermates were averaged and one value per litter was used (Zorrilla, 1997; Lazic, 2013). Estrous phase was determined daily between the hours of 07:00 and 08:30 prior to behaviour testing. A single investigator used lavage with a p200 pipette and 20 μ L of sterile physiological saline to collect cells from the vaginal wall for immediate visual examination with a light microscope. Proestrous was defined by the presence of uniform nucleated cells, while unnucleated cornified squamous cells were characteristic of estrous, densely packed leukocytes indicated metestrous, and scattered leukocytes alongside nucleated cells indicated diestrous (Hubscher et al., 2005). Estrous determination began 5 days prior to behaviour testing and continued throughout experimentation. All rats displayed a typical 4 - 5 day cycle. Additional handling included exposure to investigators and emphasized picking up and moving the rats until these motions could be carried out with ease, as well as habituation to transport between the housing and testing locations. All animal work occurred during the light phase (07:00-19:00 hrs) with the majority of behaviour testing performed between 08:30-17:00. Testing began at 8 weeks of age (young adulthood) and was completed by 15 weeks of age. The order of testing was PPI, CMOR, Sociability, Oddity Discrimination, and finally MK-801 induced locomotor activity (Figure 4.1 B). Ethanol (40%) was used to clean all behaviour testing equipment between rats.

PPI: PPI measures the percent attenuation of motor response to a startling tone when the tone is preceded by a brief prepulse (Figure 4.2 A; Lins et al., 2018). Two SR-LAB startle boxes (San Diego Instruments, San Diego, CA, USA) were used. Each session had constant background noise (70 dB) and began with 5 min of acclimatization, followed by 6 pulse-alone trials (120 dB, 40 ms). Pulse-alone (6), prepulse + pulse (36) and no stimulus (6) trials were then presented in a pseudorandom order, followed by 6 additional pulse-alone trials. Prepulse + pulse trials began with a 20 ms prepulse of 3, 6, or 12 dB above background (70 dB). Prepulse–pulse intervals (time between the onset of the prepulse and the 120-dB pulse) were short (30 ms) or long (80 ms). The inter-trial interval varied randomly from 3 to 14 s (Meyer et al., 2009; Howland et al., 2012; Ballendine et al., 2015).

Sociability Task: The testing apparatus was a rectangular arena (150 x 40 cm) of black corrugated plastic divided into three compartments, one middle start compartment (30 x 40 cm) and two ‘stranger’ compartments on either side (60 x 40 cm, see Figure 4.3 A; Henbid et al., 2017; Lins et al., 2018). The walls dividing the middle compartment from the stranger compartments were clear Plexiglas (extend 12 cm from each wall leaving a 16 cm opening allowing travel between compartments) and removable black opaque barriers which, when inserted, prevented entry into the stranger compartments. Each stranger compartment contained a circular mesh cage (18 cm diameter, 20 cm height) with hinged lid (3/4” plywood, painted matte black). The height of the cage was extended 20 cm with vertical metal rods to discourage climbing. The task began with 10 min habituation with the barriers removed. The test rat was then contained in the middle section with the barriers in place and a stranger rat was placed in one of the mesh cages. The barriers were removed, and the test rat explored for an additional 10 min. Video recording and locomotor activity tracking was done with EthoVision software, and videos were manually scored with a stopwatch by a trained investigator blind to treatment status, and the opaque cage roof obscured the location of the stranger rat. Stimulus exploration was scored when the test rat directly approached (watching, contacting, sniffing, or circling) each of the cages, with the face of the rat oriented toward the cage at a maximum distance of 2 cm. All stranger rats were sex, age, and treatment matched to the test rat (Bitanhirwe et al., 2010; Henbid et al., 2017).

MK-801 induced locomotor activity: The apparatus was a square arena (40 x 40 x 60 cm) made of black corrugated plastic (Figure 4.3 D; Lins et al., 2018). A camera mounted to the ceiling recorded all activity and EthoVision software was used to track activity. Rats were tested 4 at a time, with each rat placed in 1 of 4 separate arenas for 30 min of habituation. Immediately following, rats were administered MK-801 (0.1 mg/kg; i.p.) and placed back into the arena for an additional 120 min. Activity was recoded with Noldus Ethovision XT 11.5 software.

Visual, tactile, and CMOR: This task uses spontaneous exploratory behaviour to assess visual memory, tactile memory, and visual-tactile sensory integration (Winters and Reid, 2010; Jacklin et al., 2012). The testing apparatus was a Y-shaped maze with 1 start arm and 2 object arms (10 × 27 cm) made of white corrugated plastic (Figure 4.4; Lins et al., 2018; Paylor et al., 2018). A white plastic guillotine-style door separated the start arm from the object arms, and Velcro at the

distal end of the object arms fixed objects in place. A removable, clear Plexiglas barrier could be inserted in front of the objects. A tripod positioned above the apparatus held a video camera that recorded the task activity. Rats were habituated to the apparatus twice for 10 min. Lighting alternated during habituation between white light (during visual phases) and red light (during tactile phases) for 5 min each with the order counterbalanced, and the clear barriers were in place for one day of habituation and removed for the other with order counterbalanced. Test days consisted of a 3 min sample phase with two identical copies of an object attached with Velcro to the maze, a 60 min delay, and then a 2 min test phase with a third copy of the original object and a novel object placed in the maze. Rats began each phase in the start arm; the guillotine door was opened and closed once the rat entered the object arms. This task consisted of 3 distinct tests performed on 3 separate days in the following sequence: tactile memory (day 1; Figure 4.4 A), visual memory (day 2; Figure 4.4 C) and crossmodal memory (day 3; Figure 4.4 E). Red light illuminated the tactile phases allowing the rats' behaviour to be recorded while preventing the rats' visual assessment of the objects and the removal of the clear barriers allowed for tactile exploration. White light was used during visual phases, but clear Plexiglas barriers in front of the objects prevented tactile exploration. CMOR had a tactile sample phase (red light, no barriers) and a visual test phase (white light, clear barriers). Recognition memory was defined as significantly greater exploration of the novel object than the familiar object. Behaviour recordings were manually scored with a stopwatch by investigators blind to the treatment status of the rats and identity of the objects (Winters and Reid, 2010; Ballendine et al., 2015).

Oddity discrimination: The testing apparatus was a square arena (60 x 60 x 60 cm) constructed of white corrugated plastic with Velcro in each of the 4 corners. Two days of habituation to the arena (10 min sessions) preceded the test day. On test day, 3 identical objects made of glass or plastic and one different, or 'odd' object were fixed to the Velcro locations (Figure 4.5; Lins et al., 2018) and the rats' activity were recorded for 5 min using a video camera mounted to the ceiling. Object exploration times were manually scored using a stopwatch by an investigator blind to the treatment status of the rats (Bartko et al., 2007a). Object examination was counted when a rat's face was oriented toward the object at a maximum distance of 2 cm.

Table 4.2: Summary of the litters and offspring included in behaviour testing.

Treatment	Total Number		PPI		CMOR		Sociability		Oddity Preference		Locomotor Activity	
	Rats	Litters	Rats	Litters	Rats	Litters	Rats	Litters	Rats	Litters	Rats	Litters
Saline	36	15	36	15	22	15	22	15	22	15	14	11
PolyI:C	35	16	35	15	24	15	24	15	24	15	20	13

a) All female offspring from n=31 litters completed PPI, while all other tasks included 1 or 2 offspring per litter. The unexpected death of 1 polyI:C rat with no female littermates reduced the number of litters tested to 15. Locomotor activity n was reduced due to some rats being diverted to concurrent research. The number of offspring per litter in each task is further summarized below. Behaviour scores for littermates were averaged for a single value per litter.

<i>Treatment</i>	<i>n=5</i>	<i>n=4</i>	<i>n=3</i>	<i>n=2</i>	<i>n=1</i>
<i>Saline</i>	1	5	1	0	8
<i>PolyI:C</i>	1	0	7	1	7

b) Number of litters with n=5 or fewer offspring included in PPI.

<i>Treatment</i>	<i>n=2</i>	<i>n=1</i>
<i>Saline</i>	7	8
<i>PolyI:C</i>	9	6

c) Number of litters with n=1 or n=2 offspring in CMOR, Sociability, and Oddity.

<i>Treatment</i>	<i>n=2</i>	<i>n=1</i>
<i>Saline</i>	3	8
<i>PolyI:C</i>	7	6

d) Number of litters with n=1 or n=2 offspring tested in MK-801 Induced Locomotor Activity.

4.3.4 Statistical analyses

A between-subjects design was used, and analyses were conducted with independent samples t-tests, one sample t-tests, and ANOVAs using Statistical Package for the Social Science version 22 (IBM, Armonk, NY). Outliers were defined as having a performance metric falling more than 2 standard deviations from the mean and were removed from analysis on a case by case basis. Outliers were identified and removed prior to calculating litter averages to prevent excessive exclusion of data points. 1 saline and 2 polyI:C rats were removed from visual recognition memory, but all litters remained represented. 2 polyI:C litters were excluded from analysis for startle to the P120 tone, 1 polyI:C litter was removed from the analysis for PPI 30 ms Interval and PPI 80 ms Interval. No litters or individuals were removed from the Oddity, Sociability, or Locomotor task analyses. Estrous phase was incorporated into analysis as a covariate; however no consistent patterns were observed, potentially due to a low n of rats in each phase. Sphericity violations were accounted for using the Greenhouse-Geisser adjustment and degrees of freedom were adjusted when Levene's Test was violated. The use of one- and two-tailed tests is specified for each task. Relationships between maternal serum cytokine concentrations and long-term offspring outcomes were determined using bivariate correlations followed by a Benjamini-Hochburg adjustment to control for multiple comparisons (Benjamini and Hochberg, 1995). All data are presented as group means \pm standard error of the mean (SEM) and asterisks indicate a significant difference between groups with a 95% confidence interval ($p < 0.05$). The pound symbol (#) is used to indicate a significant difference from a chance result.

4.4 Results

The acute effects of saline and polyI:C treatment on this cohort of pregnant dams and neonatal pups have been published previously (Lins et al., 2018). Briefly, dams treated with polyI:C had reduced body weight compared to saline when followed up at 8, 24, and 48 h post treatment, but no significant change in body temperature at the same timepoints. Maternal serum collected 3 h post treatment was analyzed with ELISA for concentrations of cytokines CXCL1, CXCL2, IL-6, and TNF- α . CXCL1 and IL-6 were significantly elevated in polyI:C-treated dams. On PND1, the average offspring mass (males and females pooled) from polyI:C-treated litters

was significantly less than controls but there was no difference in litter size between the groups (Lins et al., 2018).

4.4.1 Maternal polyI:C treatment failed to significantly affect startle or PPI

Startle responses to acoustic stimuli were assessed by measuring startle alone and PPI in saline (n=15 litters) and polyI:C female offspring (n=15 litters). Startle to the 120 dB pulses alone decreased during the session (main effect of Time: $F_{(1.35,36.45)}=26.26, p<0.001$; Figure 4.2 B) but no Treatment or interaction was present. For prepulse trials with a 30 ms (short) interval, a main effect of Prepulse Intensity on PPI ($F_{(2,56)}=40.33, p<0.001$; Figure 4.2 C) was found with no effect of Treatment. Overall, PPI was greater at 12 dB compared to 3 and 6 dB ($p<0.001$). For trials with an 80 ms (long) prepulse-pulse interval, a main effect for Prepulse Intensity was found ($F_{(2,56)}=89.37, p<0.001$; Figure 4.2 D) for PPI, but no effect of Treatment and no interaction. Overall, PPI increased with louder prepulses.

Figure 4.2: There was no effect of maternal PolyI:C treatment on startle and PPI in female offspring.

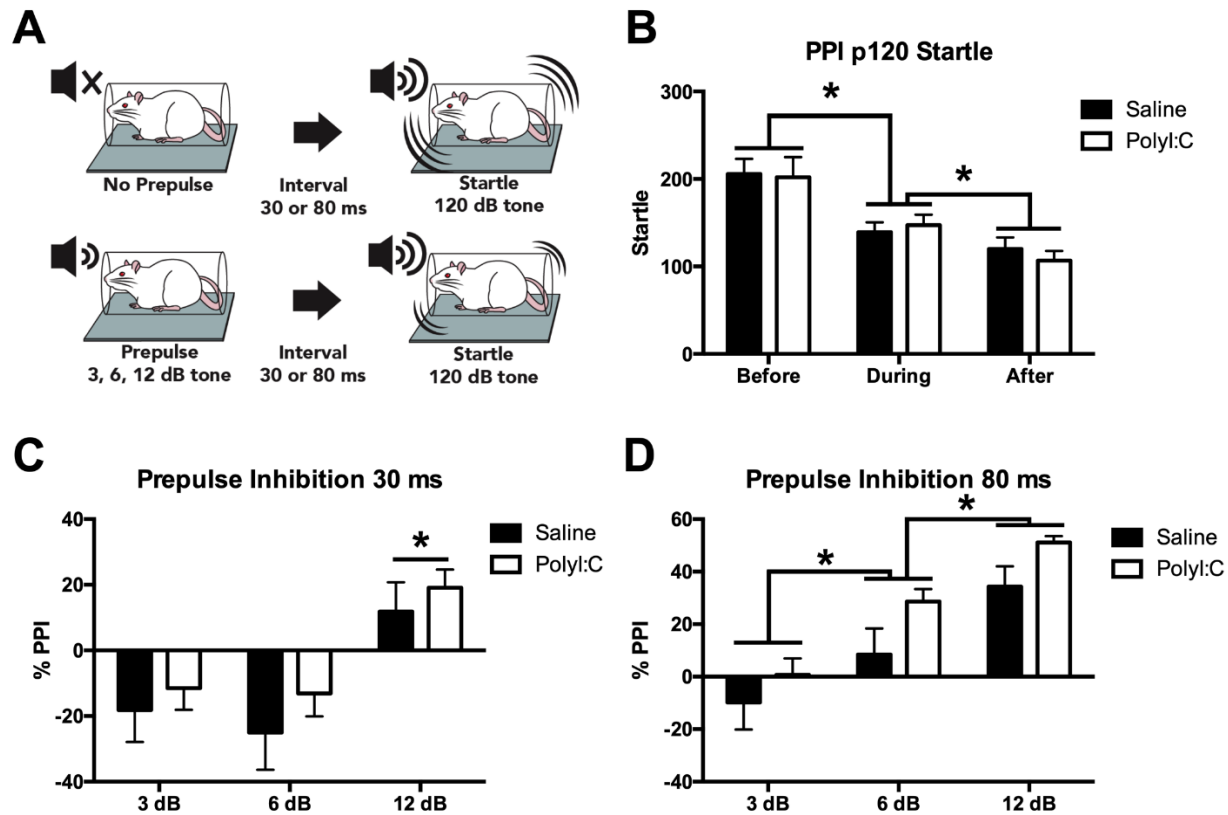


Figure 4.2: [A] Schematic illustrating a startle response to a 120-dB tone (top panel) versus the typical reduction in startle reactivity when a prepulse of 3, 6, or 12 dB precedes the startling tone (bottom panel). Schematic has been published previously (Lins et al., 2018). [B] Startle reactivity decreased over the course of the PPI testing protocol and polyI:C offspring had significantly higher reactivity at the “after” timepoint ($p < 0.05$, indicated by an asterisk, *). [C] There were no differences between groups in % PPI for the short (30 ms) prepulse-pulse interval but % PPI increased with increasing prepulse intensity where the 12 dB prepulse had higher PPI than 2 or 6 dB prepulses. [D] There were no differences between groups in % PPI for the long (80 ms) prepulse-pulse interval but % PPI increased with increasing prepulse intensity (3 dB < 6 dB < 12 dB, $p < 0.05$, indicated by asterisks, *).

4.4.2 PolyI:C offspring have sociability deficits

Both groups of female offspring (n=15 saline litters, n=15 polyI:C litters) displayed a significant preference for the cage containing an unfamiliar rat compared to the empty cage when analyzed using a within-subjects design (saline: $t_{(14)}=14.18$, $p<0.001$; polyI:C: $t_{(14)}=8.11$, $p<0.001$). When the results were compared between treatment groups, female polyI:C rats had a significantly lower discrimination ratio (DR, calculated as $\text{Exploration}_{\text{Stranger}} - \text{Exploration}_{\text{Empty}} / \text{Exploration}_{\text{Total}}$; $t_{(28)}=2.61$, $p<0.05$; Figure 4.3 B) and spent significantly more time exploring the empty cage compared to the saline controls ($t_{(28)}=-2.59$, $p<0.05$; Figure 4.3 C). There was no difference in total exploration times ($p>0.05$).

4.4.3 Both groups of offspring increase locomotor activity following MK-801 administration

Locomotor data comparing female polyI:C (n=13 litters) and saline (n=11 litters) offspring were analyzed with a mixed repeated measures ANOVA (Figure 4.3 E). Results revealed a main effect of Time ($F_{(2.47,62.24)}=62.24$, $p<0.001$; Figure 4.3 E) but no treatment effect and both groups displayed increased locomotion after MK-801 administration.

Figure 4.3: Schematic of the apparatuses for the sociability task and locomotor activity task, as well as the effects of maternal polyI:C treatment on these behaviours in female rats.

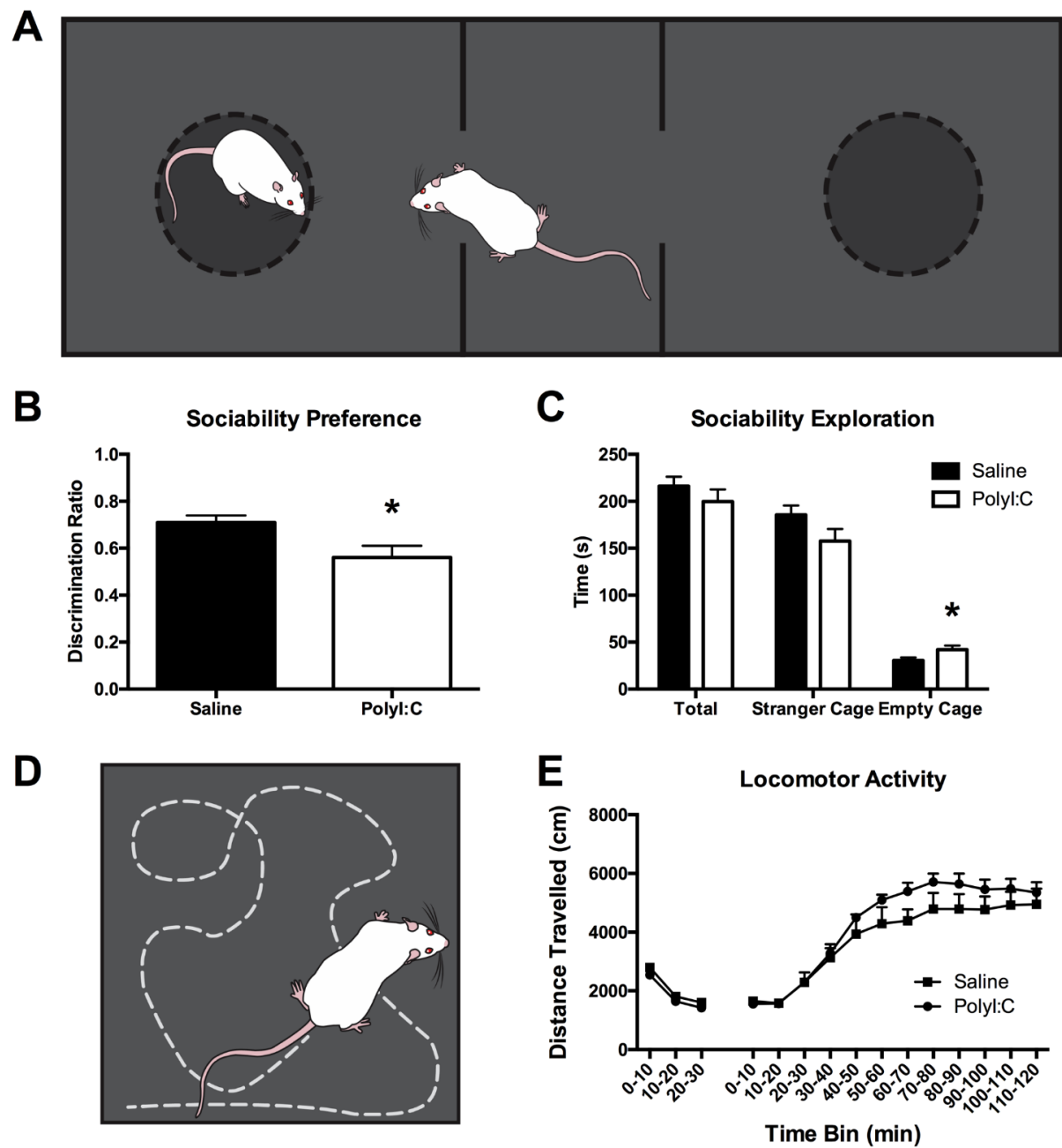


Figure 4.3: [A] Schematic representing the black, three-chambered arena used to conduct the sociability task. The chambers on either side of the center start chamber contain identical holding cages, one of which would contain a social stimulus (an age, sex, and treatment matched stranger rat) while the test rat was free to explore. Schematic has been published previously (Henbid et al., 2017; Lins et al., 2018). [B] When the exploration data is presented as a discrimination ratio, both groups show significant preference for the stranger rat; however, polyI:C offspring show significantly less preference when compared to saline offspring. [C] There was no significant difference between groups in total exploration or exploration of the social stimulus, although polyI:C rats spent more time exploring the non-social stimulus than saline rats. [D] Schematic of the black, square arena where rats' activity was monitored before and after administration of MK-801. Schematic has been published previously (Lins et al., 2018). [E] Graph displaying locomotor activity as distance travelled per 10 min time bin. Both groups had elevated locomotor activity following MK-801 administration, but there was no effect of maternal treatment.

4.4.4 PolyI:C offspring perform tactile, but not visual, object recognition memory and neither group display crossmodal recognition

All CMOR data is presented as a discrimination ratio (DR; $\text{exploration}_{\text{novel}} - \text{exploration}_{\text{familiar}} / \text{exploration}_{\text{total}}$) for the first minute of the test phase. One-tailed single sample t-tests compared each group's exploration to chance (DR of 0). Both groups demonstrated significant tactile object recognition memory (saline: $t_{(14)}=3.00$, $p<0.01$; polyI:C $t_{(14)}=11.53$, $p<0.001$; Figure 4.4 B). PolyI:C females did not perform above chance for visual memory (polyI:C: $t_{(13)}=0.49$, $p>0.05$), although saline offspring showed significant preference for the novel object ($t_{(15)}=2.72$, $p<0.05$). In the crossmodal phase, both groups of female rats failed to show a preference for the novel object (saline females: $t_{(14)}=0.46$, $p>0.05$; polyI:C females: $t_{(14)}=1.71$, $p>0.05$). There were no differences in total object exploration times between groups in any of the sample and test phases (Table 4.3).

Figure 4.4: Maternal polyI:C treatment impairs visual, but not tactile, recognition memory in female offspring. Neither group show crossmodal memory.

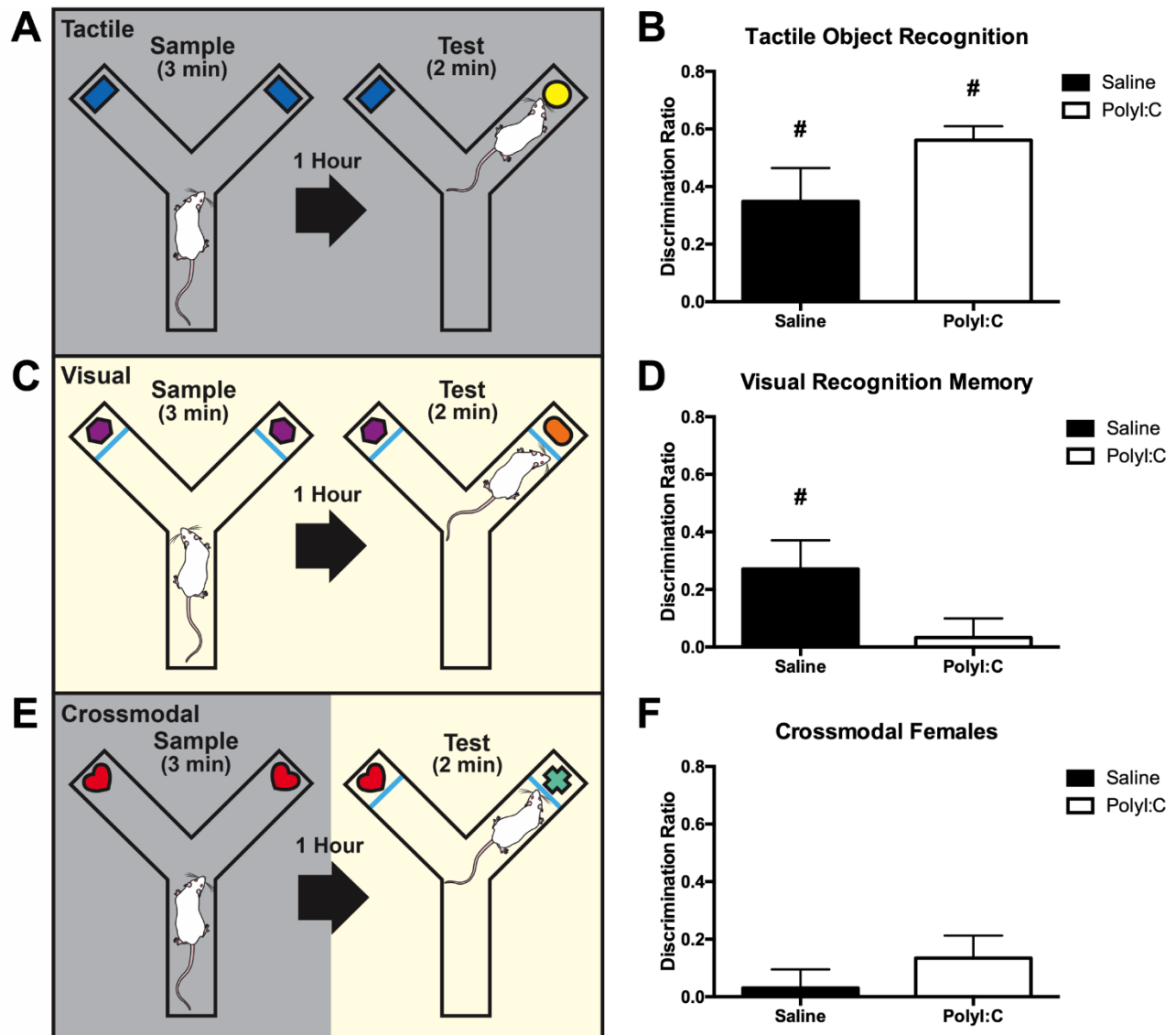


Figure 4.4: Schematic of the Y-maze arena for the CMOR task. In each sample phase there are two identical objects located at each arm of the Y-maze while the test phase uses a third, identical copy of the object from the sample phase plus a novel object. **[A]** The Y-maze assembled to conduct the tactile phase in red light conditions where the rat is able to explore objects via touch. **[B]** Both groups of offspring display robust novelty preference in the tactile phase with novel object exploration significantly greater than chance levels. **[C]** The Y-maze assembled for the visual phase which is conducted in white light conditions with the addition of a clear, plexiglass window to prevent tactile exploration of the objects, limiting the rats to visual observation. **[D]** Saline offspring demonstrated visual memory with novel object exploration significantly greater than chance but polyI:C offspring did not perform above chance levels. **[E]** The Y-maze assembled for the crossmodal phase which has a tactile sample phase and visual test phase. **[F]** Both groups failed to display crossmodal recognition memory as novel object exploration was equal to chance. The pound symbol (#) indicates significant difference from chance exploration (DR=0, $p<0.05$) in a single sample t-test. Schematic has been published previously (Lins et al., 2018; Paylor et al., 2018).

Table 4.3: CMOR task exploration.

<i>Treatment</i>	<i>Task Phase</i>	<i>Tactile</i>	<i>Visual</i>	<i>Crossmodal</i>
<i>Saline</i>	Sample	61.10 ± 2.49	6.41 ± 0.56	66.21 ± 4.73
	Test	36.71 ± 2.41	3.50 ± 0.61	3.36 ± 0.36
<i>PolyI:C</i>	Sample	60.32 ± 3.07	7.18 ± 0.83	58.61 ± 4.43
	Test	34.57 ± 3.42	2.72 ± 0.28	3.84 ± 0.27

Table 4.3: Exploration times (s) for each phase of the CMOR task, presented as $\mu \pm \text{SEM}$.

4.4.5 PolyI:C-treated offspring have reduced oddity preference compared to saline offspring

Saline (n=15 litters) and polyI:C (n=14 litters) offspring both explored the odd object at a greater than chance level (saline: $t_{(14)}=5.27$, $p<0.001$; polyI:C: $t_{(13)}=2.66$, $p<0.05$; Figure 4.5 B) when analyzed with a single sample t-test against a value of 25%. When the groups were compared directly, saline offspring spent a significantly greater % exploration with the odd object compared to polyI:C offspring ($t_{(27)}=2.24$, $p<0.05$; Figure 4.5 B). There was no difference in total exploration time between saline (97.53 ± 4.08 s) and polyI:C rats (98.94 ± 4.79 s, $t_{(27)}=-0.23$, $p<0.05$).

Figure 4.5: Maternal polyI:C treatment impairs odd object discrimination in female offspring.

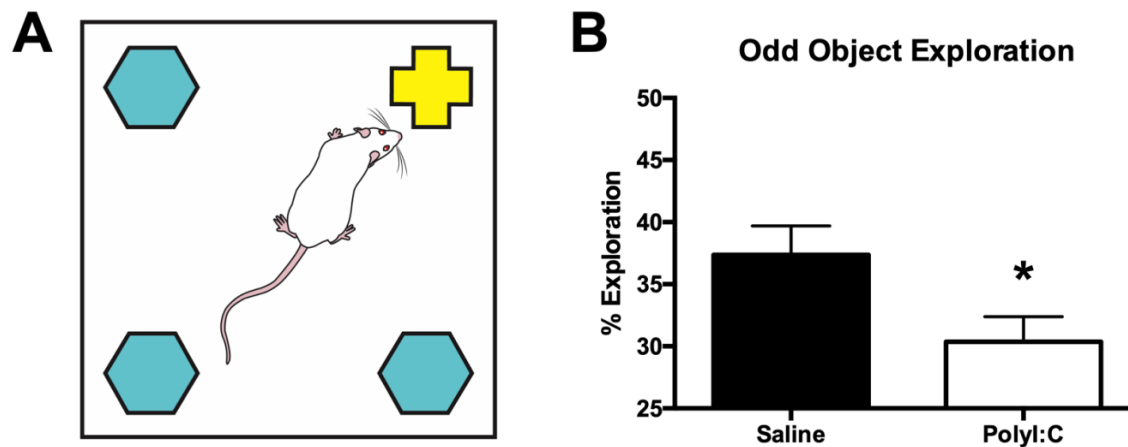


Figure 4.5: [A] Schematic of the white square arena used to conduct the oddity discrimination task showing the arrangement of three identical objects and one different, or “odd” object. Schematic has been published previously (Lins et al., 2018). [B] Bar graph displaying the percent of total object exploration spent examining the odd object. PolyI:C offspring displayed significantly less oddity preference than saline offspring ($p < 0.05$, indicated by an asterisk, *)

4.4.6 Correlations between measurements taken of the dams during pregnancy and offspring outcomes

Measurements taken during pregnancy were correlated with long-term behaviour outcomes in the female offspring. Serum cytokine concentrations of CXCL1, CXCL2, IL-6 and TNF α were determined from blood samples collected from the dams 3 h post treatment and analyzed with ELISA. Additional effects of treatment were determined through monitoring with weight and rectal temperature measurements taken 8, 24, and 48 h post treatment (Lins et al., 2018). These data were correlated to behaviour of the offspring using bivariate correlations. Maternal weight changes following treatment (anesthesia with saline or polyI:C administration and blood sampling) was the only variable associated with offspring behaviour. Greater weight loss in polyIC-treated dams 8 h post treatment was associated with reduced startle response in their female offspring during the initial tone-alone trials ($r=0.623$, $p<0.05$, B-H $p<0.05$), and this was not seen in the saline group ($r=0.44$, $p>0.05$). Weight loss in the saline dams at 24 h post treatment was correlated to lower %PPI at the 80 ms interval ($r=-0.555$, $p<0.05$, B-H $p<0.05$). Saline dam weight loss 8 h after treatment was related to the DR in the sociability task ($r=0.716$, $p<0.01$, B-H $p<0.05$). The importance of these relationships is difficult to gauge due to no significant effects of saline treatment on weight, and the lack of treatment effects in startle or PPI. Previous studies have also shown mixed results regarding maternal weight changes and offspring behaviour outcomes (Wolff and Bilkey, 2010; Vorhees et al., 2012). Dedicated studies will be necessary to determine the reliability and potential importance of these results.

4.4.7 Male and female offspring show similar behavioural profiles in response to MIA

The data presented in this paper were further analyzed in conjunction with the male littermates from Lins and colleagues (2018) with Sex and Treatment as factors using 2x2 factorial ANOVAs. It should be noted that this analysis necessitates including 2 values per litter (for each sex) which violates the assumption that subjects are independent because littermates are inherently related. No main effect of Treatment ($F_{(1,60)}=0.59$, $p>0.05$) or Sex ($F_{(1,60)}=0.25$, $p>0.05$), and no Sex by Treatment interaction ($F_{(1,60)}=0.11$, $p>0.05$) was found for 30 ms interval PPI. For 80 ms interval PPI trials, no main effect of Treatment ($F_{(1,56)}=0.10$, $p>0.05$) or Sex ($F_{(1,56)}=0.82$, $p>0.05$) was found, but a Sex by Treatment interaction was shown ($F_{(1,56)}=4.07$,

$p < 0.05$). A Tukey HSD post hoc test revealed saline females had lower PPI than saline males ($p < 0.05$). Main effects of Treatment (polyI:C offspring have a lower DR than saline offspring; $F_{(1,58)} = 9.55, p < 0.01$) and Sex (female offspring have a lower DR than male offspring; $F_{(1,58)} = 4.87, p < 0.05$) were found for sociability, with no significant interaction ($F_{(1,58)} = 0.13, p > 0.05$). Tactile object recognition memory did not differ by Treatment ($F_{(1,59)} = 0.30, p > 0.05$) or Sex ($F_{(1,59)} = 1.06, p > 0.05$); however, but a significant Sex by Treatment interaction was revealed ($F_{(1,59)} = 4.25, p < 0.05$). Tukey HSD post hoc testing failed to reveal any significant differences between individual groups. Visual object recognition memory was not affected by Treatment ($F_{(1,56)} = 3.60, p > 0.05$) or Sex ($F_{(1,56)} = 0.21, p > 0.05$), and no interaction was present ($F_{(1,55)} = 0.003, p > 0.05$). Crossmodal object recognition memory was not affected by Treatment ($F_{(1,58)} = 0.17, p > 0.05$) or Sex ($F_{(1,58)} = 0.01, p > 0.05$) and there was no interaction between these factors ($F_{(1,58)} = 3.98, p > 0.05$). A main effect of Treatment ($F_{(1,55)} = 19.30, p < 0.001$, polyI:C offspring explore the odd object less than saline offspring) was found for oddity, in the absence of a main effect of Sex ($F_{(1,55)} = 0.03, p > 0.05$) or Sex by Treatment interaction ($F_{(1,55)} = 1.88, p > 0.05$). Locomotor activity was not analyzed for Sex by Treatment interactions due to known differences in MK-801 metabolism and the use of different doses in males and females (Andiné et al., 1999).

Table 4.4: Summary of the effects of MIA on female offspring alongside the male offspring from the same cohort in a previously published companion paper (Chapter 3).

<i>Behaviour Test</i>	<i>Males</i>	<i>Females</i>
<i>PPI</i>	-	-
<i>MK-801 Locomotion</i>	↑ 47.72%	n.d.
<i>Sociability</i>	↓ 22.97% (n.s.)	↓ 21.13%
<i>Tactile Memory</i>	-	-
<i>Visual Memory</i>	↓ 90.33%	↓ 87.87%
<i>Crossmodal Memory</i>	↓ 93.71%	-
<i>Oddity Preference</i>	↓ 31.57%	↓ 18.69%

(-) = no significant change compared to controls

(n.d.) = not determined

Table 4.4: ‘↑’ indicates heightened response or facilitation while ‘↓’ indicates diminished response or impaired performance in comparison to a control group. A ‘-’ symbol indicates no significant change. Percent change was calculated as a comparison to the equivalent control group $((\text{saline} - \text{polyI:C} / \text{saline}) \times 100)$. Male locomotor data was calculated from the total distance travelled after MK-801 administration. For consistency, both male and female sociability percent change was calculated using the discrimination ratio data, although it should be noted the DR was a non-significant (n.s.) effect in the males and they instead spent significantly less time (s) exploring the social stimulus than controls.

4.5 Discussion

The adult female offspring of rat dams that received an immune stimulant during pregnancy displayed various behaviour abnormalities compared to the offspring of saline-treated dams. The polyI:C-treated offspring had reduced sociability, impaired visual discrimination, and lack of preference for an odd object compared to offspring from control litters. Both treatment groups displayed heightened locomotor activity in response to MK-801 administration and tactile recognition memory was intact in both groups. Neither group of offspring demonstrated crossmodal memory, and there were no treatment effects on PPI. These results complement a companion paper that assessed the male offspring (Lins et al., 2018), and by directly analyzing sex by treatment interactions where possible, we shown that MIA during pregnancy had similar effects on both sexes of offspring.

A significant limitation of this study is the use of timed-pregnant dams. Several studies show an impact of travel stress on the dams and offspring. For example, Moriyama and colleagues (2013) examined the effect of transport stress on seizure susceptibility in the offspring and found an increase in variability in those transported during gestation; however, maternal care behaviour had a greater impact than transport stress on seizure susceptibility (Moriyama et al., 2013). We have previously reported no observed changes to maternal behaviour following polyI:C administration (Zhang et al., 2012), but we did not assess this directly in this cohort or strain. Shipment stress also increases susceptibility to the valproate-induced developmental toxicity model of autism (Ogawa et al., 2007; Kuwagata et al., 2009). We are unable to confirm if shipment stress had a similar impact in our study. Despite these limitations, many comparable studies on development and gestational adverse events have relied on the use of timed-pregnant dams (Lodge and Grace, 2001; Du and Grace, 2013, 2016a, 2016b; Van den Eynde et al., 2014; Ballendine et al., 2015; Lins et al., 2018). Recently, Kentner and colleagues (2018) highlighted that consideration of all MIA protocols will enable comprehensive understanding of their impacts on offspring outcomes. Thus, we believe our results are of value.

4.5.1 Lack of sex-specific effects of polyI:C treatment on behaviour of the offspring

We previously reported that male polyI:C offspring from this cohort displayed greater startle to the 120 dB tone at the end of the PPI protocol compared to saline males. Although the

effect in the males was small and limited to a single parameter, the female data presented here shows no effect of MIA on any measure of PPI and acoustic startle response. The effects of MIA on PPI in rodent models are mixed with many studies showing PPI impairments in the offspring of immune challenged rats (Borrell et al., 2002; Romero et al., 2007; Wolff and Bilkey, 2010, 2008, Dickerson et al., 2010, 2013, 2014; Howland et al., 2012; Klein et al., 2013; Ballendine et al., 2015; Hadar et al., 2015) and mice (Ozawa et al., 2006; Smith et al., 2007) including impairments seen in both sexes (Meyer et al., 2009; Howland et al., 2012; Basta-Kaim et al., 2015). Other studies show no effects of MIA on PPI, similar to our observations (Missault et al., 2014; Van den Eynde et al., 2014; Vorhees et al., 2015; Lins et al., 2018). Sex effects in PPI in general have been reviewed and the influence of female sex hormone fluctuations have been studied (Kumari, 2011). High estrogen phases of the menstrual cycle have been associated with lower PPI, although this is not consistently observed (Swerdlow et al., 1997; Jovanovic et al., 2004; Kumari et al., 2008). Additionally, PPI disruption by a 5HT_{1A} agonist can be prevented by administration of exogenous estrogen and progesterone in rats which may imply a protective role of sex hormones against PPI disruption (Gogos and Van den Buuse, 2004). The results from the present study do not support the claim of a strong influence of estrous phase on PPI performance; however, it should be noted that estrous was not controlled for and the ability of the present study to detect an effect may be underpowered.

In the sociability task, polyI:C treated male offspring spent less time exploring a same-sex, unfamiliar conspecific compared to saline controls (Lins et al., 2018). We observed a different pattern of reduced sociability in the female polyI:C offspring, indicated by a significantly lower discrimination ratio compared to the saline offspring which was driven by significantly more time exploring the empty cage on the opposite side of the apparatus. The social exploration data is presented as a comparison between the saline and polyI:C offspring (comparing the relative degree of social preference), while others have presented the data as a within-subjects comparison to report either the presence or absence of social preference (Silverman et al., 2010). We believe comparing treatment groups allows the detection of subtle behaviour differences that could be missed in instances where stimuli with a substantial difference in salience (such as an unfamiliar rat versus an empty cage) result in high discrimination ratios, and this natural preference would need to be abolished to show a treatment

effect. Natural preference for social stimuli versus objects is documented in rodents (Lee and Green, 2016). Presenting the data as a between groups comparison also allows direct evaluation alongside previously published sociability data from our lab, including that of the male littermates from this cohort (Henbid et al., 2017; Lins et al., 2018). The use of single sample comparisons may be best suited to tasks that are challenging for control animals to complete, such as the complex visual discrimination tasks including visual and crossmodal recognition memory where visual stimuli are less salient and the resulting DRs tend to be lower (Winters and Reid, 2010). Both strategies of data analysis are common in behaviour literature, and factors such as strength or salience of stimuli and task difficulty should be considered when representing data. Overall, both male and female polyI:C offspring display a deficit in sociability compared to controls, but this presents in a subtly different manner depending on sex and may be related to previously observed differences in PFC development (Piontkewitz et al., 2011b; Paylor et al., 2016). The direct significance of MIA-induced developmental trajectory differences to the aberrant social behaviour observed here remains to be determined.

The effects of MK-801 administration on locomotor activity have been reported in previous studies with mixed results (Zuckerman and Weiner, 2005; Howland et al., 2012; Vorhees et al., 2012; Missault et al., 2014). The male siblings in this cohort were significantly affected by a dose of 0.2 mg/kg (i.p) indicated by heightened locomotor activity which was not seen in the control males (Lins et al., 2018). The females in this paper were given a lower dose of 0.1 mg/kg comparable to other studies (Andiné et al., 1999; Howland et al., 2012; Zhao et al., 2013). Unfortunately, both the saline and polyI:C females showed increased locomotion which confounds the ability to discern whether prenatal polyI:C treatment affected sensitivity to MK-801.

In the CMOR task, both saline and polyI:C females demonstrated object recognition in the tactile phase of the test, similar to what was seen in males of the same cohort (Lins et al., 2018) and Long Evans males (Ballendine et al., 2015). PolyI:C treated offspring were impaired in the visual phase, and neither group performed significantly different from chance exploration in the crossmodal phase, a notable distinction from the crossmodal memory exhibited by saline-treated males (Lins et al., 2018). Previous studies on MIA offspring have shown reduced discrimination in object memory behaviour tasks in females and lower discrimination ratios are

common in the crossmodal task, possibly reflecting task difficulty (Howland et al., 2012; Ballendine et al., 2015; Marks et al., 2016). Visual and crossmodal memory depend on the perirhinal cortex while the posterior parietal cortex is necessary for tactile memory, suggesting there may be regionally specific deficits as a result of MIA (Winters and Reid, 2010; Jacklin et al., 2016).

Oddity preference and perception have been assessed in several tasks using rats and mice (Bussey et al., 2005; Bartko et al., 2007a, 2007b; Cowell et al., 2010; Cloke et al., 2016; Marks et al., 2018; Paylor et al., 2018); however, to our knowledge this is the first study to assess this oddity task in female rats. Improved understanding of the nature of oddity discrimination is relevant for the successful management of cognitive impairment in conditions such as schizophrenia, a symptom domain highly related to patient functional outcomes (Cloke et al., 2016). Prenatal polyI:C treatment affected females in the same manner as males with a significant reduction in oddity preference compared to saline offspring (Lins et al., 2018). The successful performance of oddity preference depends on multisensory integration similar to CMOR, yet distinct in that visual and tactile associations can be formed simultaneously and there is no mnemonic demand in the oddity task (Cloke et al., 2016). Multisensory integration is disrupted by NMDA receptor antagonism using ketamine in the OFC and reversed with $\alpha_4\beta_2$ nicotinic acetylcholine in a GABA_A dependent mechanism (Cloke et al., 2016) and abnormalities in these brain regions and receptor types may be good candidates to explore in future studies of MIA and oddity preference.

The degree to which these behavioural effects replicate or contradict previous data varies. The MIA literature displays a lack of reproducibility, which may be due to variety of protocols used. Procedural variations in model species and strain, timing of inflammatory insult, inflammatory agent, and dose and route of administration have the potential to alter experimental outcomes. Other details such as rodent housing (bedding type, pathogen-free status, temperature, etc.), parental age, maternal experience, food and water quality, cage companions, and age at weaning, which are not commonly reported, may influence outcomes and reduce reproducibility of the model (Smolders et al., 2018; Kentner et al., 2019). The basic protocol used here is relatively common in MIA literature, yet very similar protocols yield contrasting behaviour responses; for example, hypo- versus hyperlocomotion in an open field (Van den Eynde et al.,

2014; Lins et al., 2018). Comparison in this case is complicated by the use of spontaneous vs. drug-enhanced locomotor paradigms and the collection of maternal blood samples (Van den Eynde et al., 2014; Lins et al., 2018). Indeed, distinct neuropathological alterations were noted with microglia activation in MIA offspring found in one study (Van den Eynde et al., 2014), while previous work from our group has found no changes in microglia in offspring generated in our laboratory (Paylor et al., 2016). These results support the notion that enhanced reporting of such variables is warranted and dedicated future studies should assess the effects of such procedural differences directly (Goldstein et al., 2014; Careaga et al., 2018; Kentner et al., 2019; Mac Giollabhuí et al., 2019).

4.5.2 Implications for sex differences in the maternal immune activation model

MIA caused by polyI:C administration resulted in altered behaviour in female offspring in multiple behaviour tasks including sociability, visual and crossmodal memory, and oddity preference. Overall, the present data do not provide strong evidence for sex differences in response to polyI:C treatment. Inflammation in pregnancy relates to the etiology of sexually dimorphic disorders, notably schizophrenia and autism (Davis and Pfaff, 2014; Goldstein et al., 2014; Patel et al., 2018). The lack of overt sex differences observed in the present study suggest the MIA model in rats may be limited in this respect, though others have shown more promising results in this regard (Piontkewitz et al., 2011a; Zhang et al., 2012).

CHAPTER 5

GENERAL DISCUSSION

The work in this dissertation used 3 distinct rat models to produce behaviour abnormalities characteristic of psychiatric illness such as schizophrenia in order to better understand neuropathology and disease risk factors, as well as putative treatments.

5.1 Summary of major thesis findings

1. The *l*-, but not *d*-, enantiomer of novel compound GOV reversed PPI deficits in an acute hyperDAergic model induced by systemic apomorphine, as well as an acute NMDA receptor hypofunction model induced by systemic MK-801. These data support further examination of GOV for the management of psychiatric illness.
2. PolyI:C treatment on GD15 of pregnancy results in male offspring with behaviour abnormalities in all three symptom domains of schizophrenia; positive, negative, and cognitive.
3. Female offspring displayed similar behavioural effects as their male siblings with the exception of locomotor activity where an effect in the females could not be established.
4. Serum concentrations of cytokines CXCL1, CXCL2, IL-6, and TNF α collected from pregnant dams 3h post polyI:C administration do not correlate to the severity of offspring behaviour abnormalities.

5.2 Acute and neurodevelopmental disease models

This thesis included the use of both pharmacological and neurodevelopmental models. The advantages and disadvantages of each type of model are introduced in their respective sections of the introduction. Briefly, the acute systemic administration of APO and MK-801 as pharmacological models of schizophrenia are advantageous due to their ease of use, reliable effects, and ability to link effects to the known receptor targets of each compound. The major disadvantage is a lack of etiological validity. Neurodevelopmental models such as MIA have a much better claim towards achieving etiological validity as inflammatory events in utero are well

established to increase schizophrenia risk, yet they are time consuming and therefore expensive to produce (Nestler and Hyman, 2010).

5.2.2 Validity and replication in modeling psychiatric illness

A recurring theme throughout this dissertation is achieving valid representations of complex psychiatric illness in experimental rodent models. Ideally, a model is able to achieve validity on multiple levels, notably face validity, construct validity, etiological validity, and predictive validity (Nestler and Hyman, 2010). While these concepts are frequently discussed in the context of animal models, they are also used to assess behaviour tasks (Young et al., 2009). The extent to which the models used in these studies achieve validity have been discussed throughout the general introduction with the general conclusion that the MIA model's inclusion of an etologically-relevant risk factor and a neurodevelopmental time course contribute to greater overall validity than the acute pharmacological models.

A notable drawback of the MIA model is poor reproducibility. Reproducibility is important in research. The inability to reproduce an effect generally introduces doubt surrounding the original findings or suggests a failure in methodological rigor either on the part of the original investigators in outlining their procedures or the subsequent researchers' ability to reproduce the conditions necessary for success. The lack of reproducibility following MIA is concerning, though perhaps not surprising. As discussed in Chapter 3, and reviewed by Kentner and colleagues (2019), there has been little standardization in the procedures used to generate MIA offspring and there are many opportunities for procedural variations. Additionally, the impacts of factors typically out of the investigators' control, such as the pathogen-free (or not) status of the animal care facilities, cage dimensions/materials, and others. Across laboratories, numerous procedural differences are reported, many of which are known to influence behaviour outcomes (Kentner et al., 2019). Various laboratories use different species and strains of rats and mice, and differing behaviour results are often seen across strains (Swerdlow et al., 2000). The timing of administration of the inflammatory agent influences which developmental processes are most vulnerable to disruption, and while some timepoints are more commonly used in generating MIA models, other timepoints are used as well and distinct offspring behaviour outcomes are reported based on earlier or later exposures. The inflammatory agent used also

varies, with polyI:C the most commonly seen but the use of LPS and influenza virus are also seen, while other studies administer specific cytokines (Smith et al., 2007; Chlodzinska et al., 2011). Further still, the use of different manufacturers or molecular weights of polyI:C may also result in distinct behaviour outcomes in the offspring (Kentner et al., 2019).

Despite the presence of many confounding factors resulting in poor replication within the literature, it could be argued this contributes to validity of the MIA model. A recent study that examined clinical populations found timing of gestational insult and offspring sex were relevant in the psychopathological phenotypes observed in the offspring during childhood (Mac Giollabhui et al., 2019). The variability seen across animal studies, likely due to procedural variations, may create conditions representative of the diverse circumstances that contribute to the development of complex human psychiatric illness, implying that validity can exist despite poor reproducibility. Overall, the MIA model is vulnerable to circumstances that have increased its propensity for poor reproducibility, yet these procedural variations could reveal an immense amount of information surrounding the impact of environmental risk factors in the development of psychopathology (Kentner et al., 2019).

5.2.3 Modelling psychiatric illness in the research domain criteria era

A recent shift in the conceptualization of psychopathology is changing the way psychiatric illness is classified. The traditional approach detailed in the DSM-5 is based on the practice of using clinical observations to classify clusters of symptoms into an established nosological category; for example, defining a patient as having schizophrenia and excluding other diagnoses such as bipolar disorder. A limitation of this approach is the common occurrence of comorbid psychiatric and mood disorders. As reviewed by Sanislow and colleagues (2010), the decision that co-occurring symptoms in a patient should be attributed to multiple conditions as opposed to a single, or related, underlying mechanism is contentious. This is exemplified by the heterogeneous diagnostic criteria for schizophrenia; two patients with entirely different symptoms can receive the same diagnosis. In a seeming contradiction, criteria also overlap between conditions, so two patients with the same symptom may receive different diagnoses (Sanislow et al., 2010). This is significant because a common mechanism may be involved in several disorders and several mechanisms may be involved in one disorder, yet the DSM-5 fails

to consider these scenarios. Perhaps the most consequential limitation of the conventional diagnostic approach is that it emerged solely from clinical observation, isolated from preclinical neuroscience research. In turn, preclinical neuroscience research has approached psychopathology in the context of complex clinical diagnoses that have not been integrated with preclinical findings and that are challenging, if not impossible, to observe in non-human animals. The current paradigm of separation of clinic from research may be hindering advancement of the field (Sanislow et al., 2010). The emerging alternative is a classification system proposed by the National Institute of Mental Health (NIMH) known as the Research Domain Criteria (RDoC) initiative. RDoC proposes the use of transdiagnostic constructs that underlie the core psychopathological mechanisms of mental illness with the goal of improving the integration of preclinical behavioural neuroscience and clinical practice (Sanislow et al., 2010; Shankman and Gorka, 2015).

RDoC has outlined five domains of psychological processes with the goal to relate these to biological mechanisms that explain psychiatric symptoms; Arousal/Regulatory Systems, Negative Valence System, Positive Valence System, Systems for Social Processes, and Cognitive Systems. Behavioural, genetic, and neurophysiological research were considered in defining these domains (Sanislow et al., 2010; Shankman and Gorka, 2015). The intentionally broad domain categories are further divided into constructs and sub-constructs (Shankman and Gorka, 2015).

The use of animal models within the RDoC framework, and arguably other frameworks as well, will benefit from avoiding anthropomorphism of animal behaviours and instead interpret behaviour as an observable output representative of a biological process, in this case one of the 5 RDoC domains (Anderzhanova et al., 2017). Additionally, behaviour must be interpreted in a species-specific manner; traits such as excessive aggression are maladaptive for modern humans but may be adaptive in rodents and therefore not indicative of pathology (Anderzhanova et al., 2017). The RDoC era will likely have implications for how animal models are described and related to human disease.

5.3 Potential mechanisms of behaviour disruption

The primary outcomes measured throughout this thesis were behavioural abnormalities observed in the experimental treatment groups (acute APO, acute MK-801, or MIA-exposure in utero). The experiments included on offspring of polyI:C treated dams do not directly assess mechanisms of the observed behaviour abnormalities. Other research groups have explored potential mechanisms that may underlie the manifestation of the abnormal behaviour seen in inflammation-exposed offspring and pertinent findings are included below.

5.3.1 Selective deficits in interneuron populations

GABAergic interneurons are increasingly recognized as important mediators of pathology in psychiatric illness. Schizophrenia patients have reduced cortical levels of proteins glutamate decarboxylase 67; altered levels of vGAT1, a GABA transporter; and altered levels of GABA receptor subunits (Canetta et al., 2016). These abnormalities are most frequently observed in parvalbumin (PV) interneurons, a population of cells that are essential for the maintenance of cortical gamma oscillations (Bartos et al., 2007). Several lines of evidence have demonstrated rodent MIA offspring display abnormalities in PV interneuron populations, which are related to changes in behaviour. In mouse MIA offspring, inhibitory signalling between PV neurons and cortical pyramidal cells was reduced, but this was not the case for two other populations of interneurons (calretinin and somatostatin). These MIA mice were also impaired in a set-shifting behaviour task and showed increased anxiety-like behaviour in the elevated plus maze. The role of deficient PV cell populations in the emergence of a psychiatric phenotype is further demonstrated by optogenetic inhibition of PV neurons, which recapitulates these behaviour findings (Canetta et al., 2016).

5.3.2 Interleukin 17a

An objective of this thesis was to explore the relationship between maternal serum cytokine levels and offspring behaviour, with a focus on inflammatory cytokines CXCL1 and IL-6. A correlation between these cytokines and offspring behaviour was not found; however; another inflammatory cytokine called IL-17a has been investigated with regard to emergent MIA offspring behaviour abnormalities. As previously mentioned, polyI:C administration to pregnant

mice results in elevated IL-6; administration of polyI:C or IL-6 alone to pregnant mice results in offspring behaviour abnormalities; and blocking IL-6 in polyI:C-treated mice is sufficient to prevent behaviour abnormalities in MIA offspring (Smith et al., 2007). IL-6 is required for T helper cell 17 differentiation, which is followed by downstream production of IL-17a. In MIA mouse offspring that were treated with a blocking antibody for IL-17a, MIA-induced abnormalities in cortical development (such as disorganized cortical layers) were prevented (Choi et al., 2016). The abnormal cortical organization in MIA offspring is localized to the somatosensory cortex, secondary motor cortex, and temporal association area, and a reduction in PV interneurons is a notable characteristic of these abnormal cortical “patches”. Whole cell patch clamping revealed reduced miniature IPSCs in pyramidal cells, as well as increased c-fos (Shin Yim et al., 2017). These methods were not employed in this thesis work and similar effects may be seen in rats.

MIA mice display behaviour abnormalities including pups producing abnormal levels of ultrasonic vocalizations when separated from their mother, decreased social interaction, and enhanced marble burying; all behaviours considered to be associated with an autism-like phenotype. These behaviour effects were normalized to control levels in pups whose mothers were administered a blocking IL-17a antibody prior to polyI:C (Choi et al., 2016). Studies further implicated IL-17a by administering polyI:C to genetic KO mice that lack ROR γ t, a regulator of IL-17a signalling, resulting in offspring with a normal behaviour phenotype. Finally, administration of IL-17a alone is sufficient to produce offspring with disrupted laminar organization of the cortex and the same behaviour phenotype seen in MIA offspring (Choi et al., 2016). Examination of the somatosensory cortex “patches” with reduced PV cell expression revealed a relationship between patch size and the presence of ASD-like behaviour abnormalities, and KO of neuron-specific expression of IL-17a in MIA offspring prevented both patch development and behaviour abnormalities (Shin Yim et al., 2017). In the present thesis, maternal cytokine levels alone were not predictive of behaviour abnormalities, but perhaps a measure of anatomical abnormalities, such as cortical ‘patch’ size in the offspring would better predict behaviour.

5.3.3 Cerebral ventricles and potential disruption of ion regulation

Among the most reliable anatomical abnormalities seen in patients with schizophrenia are intraventricular calcifications and enlarged cerebral lateral ventricles, and this is related to maternal serum levels of IL-8 during pregnancy (Ellman et al., 2010). While anatomical abnormalities were not assessed in this thesis, enlarged ventricles have been reported in MIA rat offspring (Piontkewitz et al., 2011). The ventricles house the choroid plexus, the site of CSF production and a key region of ion and metal transport within the brain (Smith and Rapoport, 1986; Spector et al., 2015). Due to the critical role of ions concentrations in maintaining normal physiological function of neurons, disruptions in the ventricles may result in functional deficits in brain function (Lins et al., 2016). A case study assessed endogenous ion distribution around the ventricles in a MIA rat offspring with large ventricles and found increased levels of Cl^- , decreased levels of K^+ , and punctate levels of high Ca^{2+} , indicating ion transport around the ventricles may be disrupted in MIA offspring, although this effect remains to be corroborated with a higher number of replicates (Lins et al., 2016).

5.3.4 Epigenetic factors

As there is now extensive evidence to suggest psychiatric illness risk is influenced by genetic and environmental factors, epigenetics has emerged as an area of interest, and indeed changes in gene expression have been discovered in patients with psychiatric illness (Mirnics et al., 2006). One study tested MIA mice, the offspring of dams treated in pregnancy similarly to the rats in Chapters 3 and 4, and found epigenetic changes in the cortex including decreased expression of genes involved in glutamate signalling (Tang et al., 2013). Another study looked at the mPFC of mouse offspring and found genome-wide DNA methylation differences compared to controls, with differences seen when inflammation was induced on GD09 or GD17 (Richetto et al., 2017). These data indicate that MIA causes epigenetic modification of gene expression and this may contribute to the behaviour abnormalities seen.

5.3.5 Altered perineuronal nets

Other studies have assessed the effects of MIA on extracellular matrix proteins known as perineuronal nets (PNNs). PNNs preferentially surround parvalbumin-containing interneurons

and regulate synaptic plasticity. Post-mortem brain samples from patients with schizophrenia show reduced PNNs in various regions including the PFC. PNNs are particularly relevant to developmental models of psychiatric illness as their levels increase during the postnatal period with the most rapid changes occurring in adolescence, corresponding to the critical period commonly associated with onset of psychiatric illness. Rat offspring with a history of prenatal inflammation exposure had various alterations in PNN density throughout development, including decreased PNNs in young adulthood (Paylor et al., 2016). The consequences of variable changes in PNN density throughout development have yet to be fully elucidated, yet these data provide means by which an inflammatory event in early development may influence behaviour outcomes later in life.

One way to consider the models used in this thesis, namely acute effects of pharmacological agents versus the development of abnormalities after an early life insult, is through the concept of traits versus states. A trait is a persistent underlying effect that is causal, or otherwise a contributor to disease pathology, while a state is an observable manifestation of a disease, or disease-like occurrence which may be transient (Chen et al., 2006). Acute drug administration as used in Chapter 2 is effective in creating a transient psychosis-like state and, within the short time frame (20 minutes or less) between drug administration and behaviour testing, persistent changes such as altered gene regulation or PNNs have not had time to occur. In contrast, the data described above support that MIA produces persistent traits that are reminiscent of those seen in psychiatric illness. These concepts are similar to those with further discussion in section 5.2.3. Modelling psychiatric illness in the research domain criteria era, regarding the current nosology of psychiatric illnesses and how the system of diagnosing, describing, and prescribing could be improved.

5.3.6 Application of potential mechanisms to thesis behaviour tasks

The experiments in chapters 3 and 4 focused on behaviour effects in MIA offspring. The mechanisms previously outlined in this section may be applicable to understanding the behaviour abnormalities induced by maternal treatment with polyI:C. Both male and female polyI:C rats in the present studies displayed sociability deficits, similar to those reported in mice by Choi and colleagues (2016) and Shin Yim and colleagues (2017), which were reversed by blocking IL-17a

signalling and increasing inhibitory transmission in PV cell-deficient cortical patches, and similar mechanisms may be present in MIA rats.

Deficiencies in inhibitory signalling can result in enhanced DA transmission (Tse et al., 2015). The behavioural index of this effect used in this thesis is locomotor activity, which was found to be significantly elevated in male MIA offspring after systemic administration of MK-801. Due to the direct and indirect excitatory projections from the PFC to midbrain DA neurons, PFC disinhibition may result in striatal DA elevations, and hyperlocomotive behaviour (Tse et al., 2015).

Cognitive performance and sensory processing have been linked to cortical gamma oscillatory activity. These synchronized oscillations are dependent on fast-spiking, PV interneurons, the same population of cells shown to be deficient in the MIA model (Bartos et al., 2007; Canetta et al., 2016). Gamma oscillations have been proposed for involvement in sensory binding of features into unified concepts (Bartos et al., 2007), which may be applicable to performance in sensory integration tasks such as oddity discrimination and visual, tactile, and crossmodal recognition, of which several domains were shown to be impaired throughout this thesis. Deficits in GABAergic transmission have also been shown to disrupt performance in operant set-shifting (Tse et al., 2015); although it should be noted that the data presented in this thesis revealed a facilitation in this behaviour, reversal learning was impaired and inhibitory signalling is known to influence cognitive flexibility which may have contributed to the observed effects.

There is extensive literature describing evidence of cortical disinhibition in MIA offspring. The behaviour abnormalities reported in this thesis have all be associated with disrupted inhibition. While functional and anatomical aberrations were not directly assessed, the behaviour data presented herein further support the presence of deficient inhibition in MIA.

5.4 The future of treatment and prevention strategies

The currently available treatment options for psychiatric illnesses such as schizophrenia have a number of disadvantages, including high rates of treatment resistance, poor compliance or discontinuation, and severe adverse events (Lally and MacCabe, 2015). Neither FGA or SGA contribute to significant improvements in the cognitive and negative symptoms, both of which

are strongly related to patient functional outcomes, and while the adverse event profiles are different between the two main antipsychotic classes, equal rates of drug discontinuation suggest SGAs are not improved in this regard (Lieberman et al., 2005; Jones et al., 2006; Young et al., 2009). Outcomes continue to be poor with many patients experiencing long-term disability (Lally and MacCabe, 2015). In addition, all new drugs have been modelled after clozapine in attempt to produce similar efficacy with an improved profile of adverse events, yet this aim remains unfulfilled. As discussed in section 1.2.3.1, Conventional antipsychotics, the failure of this strategy to improve patient care points to a critical need for innovative approaches to disease management (Lally and MacCabe, 2015). The work outlined in this thesis may contribute to innovation in the treatment of mental illness.

The work detailed in Chapter 3 approaches the issue of insufficient treatment options in a direct manner by assessing a putative therapeutic compound in a translationally relevant behaviour task. Of the two enantiomers tested, \pm GOV's ability to reverse PPI deficits induced by acute APO or MK-801 indicate promise for possible application as an antipsychotic. The basic pharmacological profile of \pm GOV is similar to conventional FGAs due to its high affinity for D2 receptors and antagonist activity. Targeting of D2 receptors is not innovative in itself, rather it reflects early treatment strategies such as haloperidol, but the efficacy seen in this thesis with the NMDA receptor antagonist model, as well as other studies that show promise in cognitive enhancement and latent inhibition (Lapish et al., 2012, 2014) suggest GOV may have benefits that have yet to be fully characterized with the potential for superior symptom reduction over conventional drugs. The investigation of compounds like GOV that originate from a long history in traditional Chinese medicine may also spur interest in exploring similar compounds in order to develop new treatments.

Schizophrenia has been well established as a neurodevelopmental disease along with characterization of various environmental factors that increase risk, such as maternal inflammation during pregnancy. The presence of a large time frame between adverse 'priming' events in development or early life and the emergence of illness, typically in young adulthood, presents the potential for identifying individuals most at risk and introducing early interventions to lessen disease severity or even prevent the transition to overt psychosis. The ability to anticipate and prevent the occurrence of psychiatric disorders would have an immense impact on

the affected individuals and revolutionize mental health treatment. The goal of prevention requires understanding the associated risk factors in order to design treatments as well as recognize susceptible individuals (Brown and Patterson, 2011). Progress toward this aim has utilized the MIA model due to the ability to induce inflammation during pregnancy and directly compare treated and untreated groups, as well as control over the life experiences and risk factors the rodents are exposed to. Previous studies show MIA-exposed offspring begin to diverge from the typical neurodevelopmental trajectory seen in control rats around the same time that behaviour abnormalities emerge (young adulthood). Neurodevelopmental abnormalities previously reported in adults include smaller hippocampal volume and larger lateral ventricle volume. In MIA rat offspring that were treated with antipsychotics clozapine or risperidone between PND 34-47 these anatomical changes and accompanying behaviour abnormalities were prevented. These studies raise the exciting prospect that the transition to overt psychosis and chronic illness can be prevented and further research in this area promising for the future of improved management of schizophrenia and additional studies with a focus on identifying at risk populations and pharmaceutical compounds effective at preventing the emergence of psychosis have great potential clinical applications.

The strategies used and reviewed by Piontkewitz and colleagues (2012) in preclinical rat studies have not yet faced the scrutiny of clinical trials, yet they may provide a way to improve outcomes for psychiatric patients by using existing treatments differently. Specifically, this would require a shift to using antipsychotics as a prophylactic in high-risk individuals. This approach has limitations, including the need to adequately determine risk, the cost of long-term treatment, the effects of treatment in young patients, selection of which antipsychotic to use, and how long treatment is required. Further, this approach does not address the limitations of the currently available compounds, in particular the lack of effect against negative symptoms and cognitive impairment, and patients would still experience the adverse events that accompany all known antipsychotic drugs.

5.5 Limitations of the thesis research

5.5.1 Exclusive use of behaviour testing

An important limitation of the research in this thesis is the exclusive use of behaviour testing. While analysis of behaviour is critically important in models of psychiatric illness, diseases that are diagnosed and treated based on clinical observations of patient behaviour, preclinical research with animal models is amenable to further exploration in order to better understand the mechanisms of pathology (Silverman and Ellegood, 2018). The use of pharmacological agents with known targets in Chapter 2 allows some degree of certainty of the effects in the brain that result in PPI impairments, but the study of hyperDAergic and NMDA receptor hypofunction in disrupting PPI is well studied and not a novel contribution in itself. The more important and novel contribution of Chapter 2 is the effects of d_1 - and l -GOV on the disrupted PPI, notably the attenuation of the effects of APO and MK-801 by l -GOV. The discussion of Chapter 2 includes speculation of the mechanisms by which l -GOV improves MK-801-induced PPI disruptions when conventional antipsychotics do not, but this study did not test this.

In Chapters 2 and 3, maternal serum was drawn in order to test the hypothesis that maternal serum concentrations would provide insight into the behaviour abnormalities observed in the offspring as adults. Maternal cytokines concentrations were found to not correlate with the severity of the offspring's behavioural phenotype, and the lack of other biological measurements left few other parameters to relate to the offspring behaviours aside from maternal weight, temperature, and offspring birthweight. The inclusion of measurements of anatomical, cellular, or electrophysiological data may have enabled a better understanding of the effects of inflammation during pregnancy on the neurobiology that underlie the offspring's behaviour abnormalities.

5.5.2 Impact of maternal nutrition on offspring behaviour outcomes

Another limitation of the studies outlined in this thesis is the lack of consideration of maternal nutrition on the developing offspring. This concern extends to the majority of MIA research as most studies do not report maternal weight changes or food intake, yet weight loss in the polyI:C-treated pregnant dams is a reliable finding in our lab (Howland et al., 2012; Zhang et

al., 2012; Ballendine et al., 2015; Paylor et al., 2016), including the experiments presented in chapters 2 and 3. Another study reported higher cytokine levels (TNF- α) and behaviour deficits in the offspring of dams that lost weight following treatment compared to those that gained weight (Missault et al., 2014), but another study found no association between maternal weight loss in MIA dams and offspring behaviour, specifically looking at PPI deficits (Wolff and Bilkey, 2010). Similar to the literature on MIA and psychiatric outcomes, there is a body of evidence exploring the influence of maternal nutrition and various mental health outcomes in the offspring (Penner and Brown, 2007). Animal studies demonstrate that maternal malnutrition is sufficient for behaviour abnormalities in the offspring (Grissom et al., 2014). In Chapter 3, the only correlation between maternal effects and offspring outcomes that remained following the correction for multiple comparisons was that dams that lost the most weight after polyI:C administration delivered pups that were smaller on PND1, and this may be an important indicator that appropriate maternal weight gain and nutrition are implicated in the long-term behaviour effects seen in the offspring. Low birth weight in humans is associated with increased risk for various psychiatric diagnoses (Mathewson et al., 2017), although in the data presented in Chapters 3 and 4, pup size on PND1 did not have robust correlations with adult behaviour. The lack of data on maternal food intake and nutrition may have confounded the results of these experiments and could be a source of variability resulting in the poor reproducibility seen throughout the field (Kentner et al., 2019). Analysis that considers maternal nutritional state should be included in future studies.

5.6 Future directions

The understanding of the risk factors and pathology of psychiatric illness is improving, with hope for more effective treatments and even preventative strategies. The work highlighted in this dissertation supports earlier evidence that maternal inflammation during pregnancy is associated with psychiatric behaviour profiles in the offspring and demonstrated the efficacy of 1-GOV in restoring PPI when disrupted by two pharmacological approaches. These experiments can inform future research to improve the management of psychiatric illnesses.

5.6.1 The effects of GOV in neurodevelopmental models of psychiatric illness

Following the assessments of GOV's effects in normal rats as well as in acute pharmacological models as demonstrated here any by others (Lapish et al., 2012, 2014b; Ashby et al., 2015), assessing GOV's effects in etiologically valid neurodevelopmental models is an option for future progression of the field. The data presented in Chapters 2 and 3 contribute to a body of evidence that the MIA model is capable of generating rats with all three symptom domains of schizophrenia and is etiologically valid. The ability of GOV to ameliorate these abnormalities would further support its position as a putative antipsychotic drug and contribute to the search for new therapeutics, particularly those that can improve the management of negative and cognitive symptoms.

5.6.2 The ability of GOV to prevent the emergence of behavioural abnormalities in neurodevelopmental models of psychiatric illness

In addition to the goal of improved management of psychiatric illnesses, the neurodevelopmental nature of schizophrenia and the ability to identify those at greatest risk presents an opportunity for early intervention and possible prevention (Insel, 2010). Previous research using the MIA model has shown that administration of antipsychotic compounds prior to adulthood prevents the emergence of behaviour abnormalities (Piontkewitz et al., 2009, 2011b, 2012). Further investigation of the effects of GOV, and other compounds, in the MIA model could contribute to a paradigm shift in how psychiatric illness is perceived and treated with great potential to lessen the burden of schizophrenia on individuals and society (Brown and Patterson, 2011).

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